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Oral Presentations

O-1: Loss of lamin B1 results in prolongation of S-phase and decondensation of chromosome territories in the interphase nucleus of mammalian cells

Darawalee Wangsa, Jordi Camps, Martin Falke Markus Brown, Chanelle M Case, Mike Erdos, Thomas Ried

Nuclear lamin B1 (LMNB1) constitutes one of the major structural proteins in the lamina mesh. We silenced the expression of LMNB1 by RNA interference in the colon cancer cell line DLD-1 and showed a dramatic redistribution of H3K27me3 from the periphery to a more homogeneous nuclear dispersion. In addition, we observed telomere attrition and an increased frequency of micronuclei and nuclear blebs. By 3D-FISH analyses, we demonstrated that the volume and surface of chromosome territories were significantly larger in LMNB1-depleted cells, suggesting that LMNB1 is required to maintain chromatin condensation in interphase nuclei. These changes led to a prolonged S phase due to activation of Chk1. Finally, silencing of LMNB1 resulted in extensive changes in alternative splicing of multiple genes and in a higher number of enlarged nuclear speckles. Taken together, our results suggest a mechanistic role of the nuclear lamina in the organization of chromosome territories, maintenance of genome integrity and proper gene splicing.

O-2: Implementing Chromosome Confirmation Capture-seq

Joshua J. Waterfall, Wei-Dong Chen, Robert L. Walker, Paul S. Meltzer, W. Michael Kuehl

In mammalian genomes regulatory regions are often not contiguous with their targets and can be separated by great distances in the linear sequence. Furthermore, the set of regulatory regions impacting any particular genomic element can depend extensively on environmental signals, the level of differentiation, and numerous other processes affecting cell state. One method to identify regulatory regions is to map physical associations - regions that loop together in the three dimensional context of the cell. With the motivation of understanding regulation of the Myc promoter in multiple myeloma, we have implemented a Circular Chromosome Confirmation Capture-sequencing (4C-seq) protocol. We will discuss what we have learned about Myc regulation in this cancer, but primarily we will share our experience on technical aspects of the assay, both experimental and analytic.

O-3: Novel Role of Sumo-Targeted Ubiquitin Ligase (STUbL) Slx5/8 in the Proteolysis of Centromeric Histone H3 Variant Cse4

Kentaro Ohkuni, Yoshimitsu Takahashi, Nagesh Pasupala, Reuben Levy-Myers, Wei Chun Au, Alexander Strunnikov, Richard E. Baker, Oliver Kerscher, and Munira A. Basrai

Centromeric histone H3, CENP-A (Cse4 in Saccharomyces cerevisiae, CENP-A in mammals), is an essential component of the kinetochore (centromeric DNA and associated proteins) that mediates attachment to microtubules and regulates faithful chromosome segregation. Overproduction of CENP-A causes ectopic mis-localization to chromosome arms and is associated with many human cancers. In S. cerevisiae, E3 ubiquitin ligase Psh1 controls the cellular levels of Cse4 to prevent its mis-localization via ubiquitin-mediated proteolysis. Post Translational Modifications (PTMs) of canonical and histone variants by phosphorylation, methylation, acetylation, ubiquitination, and sumoylation, regulate chromatin structure, gene expression and maintain genome stability. Phosphorylation, methylation, and ubiquitination of Cse4 in S. cerevisiae have been reported previously. Sumoylation of Cse4 has not been reported so far and hence we investigated whether Cse4 is sumoylated. We have shown that Cse4 is sumoylated by SUMO (Small Ubiquitin-related MOdifier) E3 ligases Siz1 and Siz2 in vivo and in vitro. Defect in sumoylation of Cse4 in siz1ÃČÂĘ siz2ÃČÂĘ strain results in the stabilization of Cse4, suggesting that SUMO modification of Cse4 regulates its degradation.

Ubiquitination of SUMO modified substrates by Sumo-Targeted Ubiquitin Ligase (STUbL) regulates proteolysis of several substrates. STUbL proteins Slx5/8 have been recently reported to have a role in centromere function. Hence, we investigated the role of Slx5/8 in the proteolysis of Cse4. Deletion of SLX5 or SLX8 results in defects in Cse4 ubiquitination and this contributes to the stabilization of Cse4 in vivo. Interestingly, deletion of SLX5 leads to the accumulation of sumoylated Cse4. Given that Slx5 is an E3 ubiquitin ligase that mediates Cse4 ubiquitination, we examined whether the loss of both E3 ligases, Slx5 and Psh1, would further stabilize Cse4. As expected, protein stability of Cse4 is highly increased in slx5ÃČÂĘ psh1ÃČÂĘ strain. Defects in Cse4 proteolysis contributes to its mis-localization as we observed enrichment of Cse4 in the chromatin fraction of slx5ÃČÂĘ strain and this was further enhanced in slx5ÃČÂĘ psh1ÃČÂĘ strain. Taken together, we conclude that Slx5/8 regulates the ubiquitin-mediated proteolysis of Cse4 in a Psh1-independent manner and that Slx5/8 prevent mislocalization of Cse4 into non-CEN DNA. We propose that the role of Slx5/8 in genome stability may be evolutionarily conserved and suggest that the chromosome segregation defects reported recently for a knockdown of RNF4 (human orthologue of Slx5/8) in human cells may be due to mis-localization of CENP-A. Our current research is focused on the identification of SUMO sites in Cse4, the role of these modifications in proteolysis of Cse4 and understanding how mis-localization of Cse4 contributes to chromosome segregation errors in yeast and human cells.

O-4: Novel role of Dbf4-Dependent Kinase in ubiquitin-dependent proteolysis of Cse4/CENPA in S. cerevisiae

Lars Boeckmann, Levi Bursch, Valerie Garcia, Sara Azeem, Wei-Chun Au, Michael Costanzo, Michael Weinreich, Charlie Boone and Munira Basrai

The evolutionarily conserved centromeric histone H3 variant Cse4/CENPA is essential for faithful chromosome segregation. CENPA overexpression and mis-localization is observed in various cancers and is particularly associated with the most aggressive cases with a poor prognosis. Consistent with this, development of aneuploidy due to Cse4/CID/CENPA overexpression has been reported in yeast, flies and humans. Given the poor prognosis for patients with aneuploid and CENPA-overexpressing (CENPA-OE) tumors, novel treatment is needed to specifically target these tumors. Currently, one of the most promising and novel approaches for selectively treating cancerous cells is to target synthetic lethal (SL) partners of mutated or mis-regulated genes. The aim of this study is to identify novel therapeutic targets for CENPA-OE tumors by identifying SL interaction partners, an approach that has not been exploited so far for these tumors. We used budding yeast as a model to perform a genome-wide screen for gene mutations/deletions that are SL in a strain overexpressing Cse4 but not in a control strain without Cse4 overexpression. This screen identified 102 temperature sensitive alleles of essential genes and 273 deletions of non-essential genes that exhibited SL with overexpression of Cse4. Five alleles of cdc7 and dbf4 were amongst the top eight hits as the most significant SL interaction partners. Cdc7 is an evolutionary conserved, Dbf4 dependent kinase (DDK), which is essential for DNA replication and has recently been shown to localize to centromeres. We validated the SL interaction of cdc7 and dbf4 with Cse4 overexpression using growth assays. Biochemical analysis showed that stability of Cse4 is increased in cdc7-7 and dbf4-1 mutants. Consistent with this, ubiquitination of Cse4 is reduced in a cdc7 mutant. ChIP and chromatin fractionation experiments showed enhanced mis-localization of Cse4 to non-centromeric chromatin in cdc7 and dbf4 mutants. Furthermore, in vitro kinase assays showed that DDK phosphorylates Cse4. These studies, supported by additional genetic analysis, revealed a novel role of DDK in regulating Cse4 levels and preventing it from mis-localizing to non-centromeric chromatin. Interestingly, inhibitors of Cdc7 are currently being used for cancer treatment in clinical trials. Our results suggest that Cdc7 inhibitors may be especially effective in the specific treatment of CENPA-OE tumors. Taken together, our studies have led to the identification of potential therapeutic targets for CENPA-OE tumors and provide novel insights into the mechanistic role of DNA replication factors in regulating Cse4 levels.

O-5: Spontaneous Mutations of Bcor and Jak1/2 Genes Lead to an Aggressive Leukemia of B-1 Progenitor B Cells

Sheryl M Gough, Liat Goldberg, Marbin Pineda, Robert L Walker,Yeulin J Zhu, Sven Bilke, Paul S Meltzer and Peter D Aplan

NUP98 gene fusions, generated by non-random chromosomal translocations, are associated with a wide spectrum of high risk hematologic malignancies and have been shown to alter normal hematopoietic stem and progenitor cell (HSPC) gene expression programs. A recurrent t(11;17)(p15;p13) translocation in patients with AML leads to the production of a NUP98ÃČÂŘPHF23 (NP23) fusion gene. The consequent NP23 fusion protein retains the PHD domain, known to bind H3K4me3, and is thought to have aberrant chromatin regulation properties. We have generated a transgenic mouse model of the NUP98-PHF23 gene fusion which develops a range of hematologic malignancies, most commonly pre-T LBL and AML. However, approximately 10% of NP23 mice develop an aggressive B-1 progenitor acute lymphoblastic leukemia (pro B-1 ALL). B-1 and B-2 lymphocytes have distinct developmental pathways and are thought to represent arms of the innate and adaptive immune systems, respectively. Mature B-2 lymphocytes predominate in the peripheral circulation, and are characterized by expression of B220; whereas B-1 lymphocytes are more prevalent in the pleural and peritoneal cavities, and do not express B220. Murine B cell malignancies typically stain positive for B220, and represent transformed B-2 cells. In the present study, NP23 progenitor ALLs displayed an immunophenotype (Lin-B220- CD19+ AA4.1+) that was identical to that of the recently described B-1 progenitor cell. All B-1 progenitor ALLs exhibited clonal rearrangements of the IgH gene locus. Specifically, these rearrangements involve favored usage of 3ÃČÂŢ VH regions, similar to observations with fetal B-1 progenitor cells, further supporting the notion that these are leukemias of B-1 progenitors. Using whole exome sequencing, we found acquired mutations in the BCL6 interacting corepressor (Bcor) gene in 5 out of 7 B-1 progenitor leukemias. The mutations were all frame shift or nonsense mutations, and were located within a 9 bp ÃČÂŠhot spotÃČÂŞ in Bcor exon 8. In addition, 4 of 7 cases had somatic mutations of Janus kinase 1 (Jak1) or 2 (Jak2), and 7/7 cases showed hyperphosphorylation of Stat3 or Stat5, consistent with the contention that the Jak1/2 mutations are activating mutations, and leading to a hypothesis that the NP23 pro B-1 ALLs which do not harbor Jak1/2 mutations may have acquired an unidentified mutation in the Jak-Stat pathway. Of note, Jak1/2 mutations have previously been identified in a subset of high-risk pediatric B-cell precursor ALL patients. The striking correlation between Bcor and Jak1/2 mutations, occurring specifically in a subset of NP23 leukemias, implies that these three mutations (NP23, Bcor, and Jak1/2) collaborate and provide the oncogenic setting for B-1 progenitor transformation.

O-6: Targeting Oncogenic RAS Mutations in Rhabdomyosarcoma

Marielle E. Yohe, John F. Shern, Berkley E. Gryder, James P. Madigan, Young K. Song, Jun S. Wei and Javed Khan

Rhabdomyosarcoma (RMS) accounts for 3% of childhood cancers with 350 new cases per year in US. It is sub-divided into two major genetic and histologic subtypes: embryonal (ERMS), which is characterized by point mutations in members of the RAS pathway and alveolar (ARMS), which is characterized by the chromosomal translocation (t2:13) that results in expression of the PAX3-FOXO1 fusion oncogene. RMS likely represents a malignant tumor of myoblast-like cells failing to exit the cell cycle and differentiate. The 5-year survival rate for patients with relapsed RMS is poor (17%), and novel treatment approaches are needed for these patients. RAS proteins, binary molecular switches capable of binding to downstream effectors only when bound to GTP, are constitutively GTP-bound as a result of missense mutation in many human cancers. NRAS, in particular, is mutated in hematologic malignancies and melanoma, and is the most common RAS family member mutated in ERMS tumors. RAS-mediated oncogenesis occurs through the inappropriate activation of signal transduction pathways that ultimately cause cell growth, cell division and the inhibition of apoptosis. The best characterized of these signal transduction pathways downstream of GTP-bound RAS are the MAP kinase pathway and the PI3 kinase pathway. Due to the high affinity of RAS proteins for GTP, the multitude of downstream RAS effectors, and the ubiquitous development of secondary resistance to

targeted agents, development of small molecules to target mutant RAS is challenging. In the current study, we use human ERMS cell lines expressing mutant RAS isoforms, namely RD (NRAS Q61H) and CTR (HRAS Q61K) to demonstrate that oncogenic mutation in RAS is necessary for ERMS tumorigenesis. Overexpression of mutant RAS isoforms in C2C12 myoblasts inhibits myogenic differentiation. The MAP kinase pathway is an important mediator of both the inappropriate proliferation and the differentiation blockade in ERMS cells expressing mutant RAS isoforms, since pharmacologic inhibition of this pathway with trametinib, an FDA-approved MEK1/2 inhibitor, leads to decreased cell proliferation, induction of apoptosis, cell cycle arrest and increased expression of myosin heavy chain, consistent with induction of myogenic differentiation of these ERMS cells. Gene expression analysis reveals that RD treated with trametinib undergoes dramatic reprogramming. In addition, trametinib displays additive effects on ERMS cell viability with cytotoxic chemotherapeutic agents such as topotecan, and synergistic effects with other targeted agents such as the pan-AKT inhibitor, MK-2206. These studies highlight the importance of oncogenic RAS mutation in ERMS tumor development and show that oncogenic RAS is an attractive target for inhibition in patients with metastatic or relapsed ERMS.

O-7: miR-215 represses BMI1 expression to promote differentiation and limit self-renewal in colorectal cancer stem cells

Matthew Jones, Toshifumi Hara, Sven Bilke, Yeulin Zhu, Walter F. Bodmer, and Ashish Lal

Since the initial description of cancer stem cells (CSCs) as a self-renewing subpopulation of malignant cells with tumor-initiating capacity, a growing body of evidence has supported the existence of CSCs in virtually every tumor type. Our previous work in colorectal cancer has identified the transcription factor CDX1 as a key regulator of CSC dynamics. The expression pattern of CDX1 in the normal colon forms a gradient with its minimum in the crypt base stem cell niche, increasing to a maximum in the mature enterocytes lining the lumen, where CDX1 transcriptionally activates genes important for enterocyte structure and function. CDX1 expression is frequently lost in colorectal cancer, resulting in more aggressive, poorly differentiated tumors with higher proportions of CSCs. These CSCs share many genetic markers with normal stem cells, including BMI1, a polycomb repressive complex 1 component involved in epigenetic silencing of genes that oppose self-renewal. Many miRNAs have been implicated in tumor suppression and carcinogenesis, but the roles of miRNAs in differentiation, particularly in colorectal cancer, remain poorly understood. We began by identifying miRNAs downstream of CDX1, reasoning that they may be effectors of differentiation. We used high-throughput small-RNA sequencing to profile miRNA expression in two pairs of colorectal cancer cell lines: CDX1-low HCT116 and HCT116 with stable CDX1 overexpression; and CDX1-high LS174T and LS174T with stable CDX1 knockdown. Validation of candidates identified by RNAseq in a larger cell line panel revealed miR-215 to be most significantly correlated with CDX1 expression. CDX1 ChIP-qPCR and promoter luciferase assays confirmed that CDX1 directly transactivates miR-215 transcription. miR-215 expression is depleted in FACS-enriched CSCs compared to unsorted samples. Overexpression of miR-215 in poorly-differentiated, highly clonogenic cell lines causes growth arrest and a dramatic decrease in colony formation. miR-215 knockdown using a miRNA sponge causes an increase in clonogenicity and impairs differentiation in CDX1-high cell lines. Results of microarray studies following miR-215 overexpression indicate that miR-215 induces terminal differentiation associated growth arrest, due in part to direct silencing of BMI1 expression and de-repression of BMI1 target genes including CDKN1A. Our work situates miR-215 as a link between CDX1 expression and BMI1 repression that governs differentiation in colorectal cancer.

O-8: Identification and characterization of non-coding RNAs transactivated by p53

Murugan Subramanian, Matthew F. Jones, Xiao Ling Li, Kannanganattu V. Prasanth, and Ashish Lal

The tumor suppressor p53 functions as a master regulatory transcription factor that controls the expression of a myriad of genes involved in diverse cellular processes including cell cycle arrest and apoptosis. Recently, several studies highlighted long non-coding RNAs (lncRNAs) as powerful players in cancer pathogenesis and

it is increasingly clear that p53 regulates the expression of a number of lncRNAs. However, only a few have been functionally characterized for their role in the p53 regulated pathway. Using cDNA microarrays we have identified the up-regulation of eight novel transcripts in three colorectal cancer cell lines, HCT116, RKO, and SW48 upon activation of p53 by Nutlin. Assessment of the protein-coding potential of these transcripts by Coding Potential Assessment Tool (CPAT) indicated that these are non-coding RNAs. Interestingly, LOC643401 lncRNA expression levels are correlated with wild-type p53 status, and its expression was 90% reduced in p53 null cells. We therefore selected this lncRNA for further characterization. We demonstrated by chromatin immunoprecipitation and promoter luciferase assays that LOC643401 is a direct transcriptional target of p53. Furthermore, we identified a putative p53-binding motif comprising two consecutive half-sites located in the LOC643401 promoter region at nucleotides -99/-119 relative to the predicted transcriptional start site. Using a promoter luciferase reporter assay and targeted deletion, by site-directed mutagenesis, of 20 nucleotides within the -99/-119 bp region of the LOC643401 promoter we identified the p53 response element responsible for transactivation of this gene by p53. Importantly, by applying a CRISPR/Cas9 system, we have also generated mutation in, or deletion of, this p53-binding motif in the promoter region of LOC643401 in HCT116 cells. Both mutation and deletion of this p53-binding motif results in total absence of the LOC643401 lncRNA. In addition, LOC643401 lncRNA expression was not induced upon activation of p53 by Nutlin or by treatment with doxorubicin. Preliminary data also suggests that, LOC643401 lncRNA deficient HCT116 cells are highly sensitive to doxorubicin-induced DNA damage with an accumulation of cells in the sub-G1fraction of the cell cycle, compared to parental HCT116 cells. This suggests that the up-regulation of LOC643401 lncRNA by p53 is necessary for cell survival in the presence of DNA damage. Taken together we have identified several p53 regulated novel lncRNAs and functional characterization of these RNAs in cancer cells will provide a better understanding of lncRNA-mediated regulation of the p53 pathway.

O-9: Dear Cancer, please "TelME" about your telomeres?

Abaan OD, Waterfall J, Davis SR, Gomez A, Edelman DC, Savage SA, Pommier Y, Meltzer PS

Telomeres play an essential role in maintaining the fidelity of chromosome ends during replication, but without an active telomere maintenance mechanism, their length decreases with each successive cell division. Replicative immortality is a hallmark of cancer and tumor cells circumvent cellular crisis associated with telomere shortening via two main mechanisms, activation of telomerase or the Alternative Lengthening of Telomeres (ALT) process. Therefore the lengths of the telomeres point to a critical aspect of tumorigenesis. Advances in next-generation sequencing (NGS) enable researchers to understand tumorigenesis in more detail then ever. Targeted-resequencing, such as whole exome sequencing (WES), is becoming a tool for molecular diagnostics and gene discovery. While WES contains data from the targeted region, the substantial amount of data from the off-target regions is not utilized for analysis. These off-target sequences likely contain additional information that could be useful for research and clinical classification. In this work, we describe a new algorithm called ÃČÂŠTelMEÃČÂŞ that can estimate relative telomere length (RTL) using any targeted-resequencing data available. TelME overcomes the targeting bias by using an internal normalization to compensate for the variation in capture-efficiency from sample-to-sample. For proof-of-principle, we used the WES data from a panel of osteosarcoma cell lines. Mesenchymal tumors rely on the ALT more then epithelial tumors, so this dataset should contain both hTERT activation and ALT resulting in a wider range of telomere lengths. RTL calculated by the algorithm for the osteosarcoma panel demonstrate correlations between lower values with hTERT expression and higher values with C-Circles positive cell lines, indicative of ALT thereby longer telomeres. In order to validate the algorithmÃČÂŢs robustness, we have utilized the previously published WES data for the NCI-60 panel of cell lines. The results suggest that most cells display the classic telomere shortening, while a few cell lines, like LOX-IMVI, have very long telomeres. These findings were validated by alternative telomere measuring assays. In summary, we believe TelME will enable utilization of otherwise overlooked off-target data generated by targeted-resequencing based molecular diagnostic tests and enable researchers to extract clinically relevant data with no additional cost or analysis time.

O-10: Transcriptome characterization by RNA sequencing identifies molecular and clinical subgroups in high risk Neuroblastoma

Shile Zhang, Jun S. Wei, Rajesh Patidar, Young K. Song, Sivasish Sindiri, Xinyu Wen, and Javed Khan

Background/objectives: Neuroblastoma (NB) is characterized with its heterogeneous clinical and biological behavior. Despite improvement of survival rate with multimodal chemo- and immunotherapy, high mortality and morbidity is still substantial for patients with metastatic disease. Previous studies of DNA sequencing characterized the genetic basis of the disease and revealed a very low somatic mutation burden of 0.60 per Mb and surprisingly few recurrently somatic mutated genes comparing to common adult solid tumors. Therefore, we hypothesize that the expressed transcriptome of the tumor cells may give insights to the tumor biology of neuroblastoma. Methods: In order to characterize the underlying NB transcriptional landscape as an integral part of the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) project, we have performed RNA sequencing in a cohort of 150 patients. To identify the important transcriptional event for NB tumors, we perform correlation analyses between clinical parameters and the sequencing data (RNA-seq and whole exome sequencing (WES) data available for 106 of the 150 patients cohort). Results: On average, we found 12.5% of the human genome is expressed in NB tumor RNA, which include 63.7% of exon, 18.0% of intron, and 4.6% of intergenic regions. Gene expression profiles showed four molecular subgroups of NB tumors with distinct survival tendency (log-ranked p=0.002). For expressed mutations, 62% of somatic mutations identified by WES was present (>10% VAF) in the RNA transcriptome when the locus is expressed (10X). We also checked for known mutated sites reported in other tumor studies, and found a known somatic mutation in TP53 p.R243W only detectable by using both RNA and DNA sequencing data. In addition, a recurrent mutation of MED12 p.N1845T was detected in 19 RNA samples (13% prevalence) by this approach, whereas it was only detected in 1 DNA sample by WES. The cause of false negatives was possibly due to low coverage of this genes in WES. Interestingly, the mutation status of MED12 p.N1845T was also significantly correlated with survival (p=0.02). Conclusions and Future directions: Our findings suggest the utility of RNA-seq in identification of mutations as biomarkers to stratify NB patients and hopefully to guide the targeted therapies. With this clinically well annotated cohort, we will continue to assess the expressed mutations, fusion genes, mRNA expression, and splicing profiles to provide clinically relevant classification and offer insight into the tumor biology of neuroblastoma.

O-11: Inhibition of the splicing of the nascent EWS-FLI transcript reverses fusion transcription factor driven oncogenic expression in Ewing Sarcoma

Suntae Kim, Patrick J. Grohar, Sara Haddock, Konrad Huppi, Carleen Klumpp, Eugen Buehler, Lee J. Helman, Scott E. Martin, Natasha J.Caplen.

Inhibition of the splicing of the nascent EWS-FLI transcript reverses fusion transcription factor driven oncogenic expression in Ewing Sarcoma Suntae Kim1, Patrick J. Grohar2, Sara Haddock1, Konrad Huppi1, Carleen Klumpp3, Eugen Buehler3, Lee J. Helman4, Scott E. Martin3, Natasha J.Caplen1. 1Gene Silencing Section, Genetics Branch, CCR, NCI; 2Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, TN; 3Trans-NIH RNAi Facility, Division of Pre-Clinical Innovation, NCATS; 4Molecular Oncology Section, Pediatric Oncology Branch, CCR, NCI Oncogenic fusion transcription factors drive development of many cancers including the bone tumor Ewing sarcoma (ES). Using a reporter assay of EWS-FLI1 activity and genome-wide RNAi screening we have identified specific RNA processing proteins required for expression of EWS-FLI1, the oncogenic fusion transcription factor associated with 85% of ES tumors. RNA processing genes identified by RNAi screening included those encoding several components of the core spliceosome complex, such as SF3B1; the non-core splicing factor, HNRNPH1; and the transcriptional regulator, SUPT6H. Follow-up analysis of the transcriptome of TC32 cells, a well-characterized ES cell line, in which HNRNPH1, SF3B1, or SUPT6H had been silenced showed decreased expression of EWS-FLI1 and reversal of EWS-FLI1 driven expression. In particular we observed the altered expression of genes involved in cell cycle, DNA replication, and regulation of transcription. To investigate the mechanistic basis of this observation we assessed the effect of HNRNPH1, SF3B1, or SUPT6H loss-of-function (LOF) on EWS-FLI1 expression in additional

ES lines. We tested SKNMC cells that harbor a similar translocation as TC32 cells, fusing intron 8 of EWSR1 to exon 6 of FLI1, and we tested two cell lines, EW8 and TC71, with breakpoints fusing exon 7 of EWSR1 to exon 6 of FLI1, and a RD-ES cells with a breakpoint that fuses exon 7 of EWSR1 to exon 5 of FLI1. Consistent with our observations in TC32 cells, HNRNPH1 LOF in SKNMC cells mediated a substantial decrease in the expression of EWS-FLI1, but silencing of HNRNPH1 had minimal effects on EWS-FLI1 expression in the other ES cell lines tested. None of the additional ES cell lines were as susceptible to the silencing of SUPT6H as TC32 cells, but we did observe that all of the ES cells were vulnerable to the loss of SF3B1. To determine how HNRNPH1 and SF3B1 process nascent EWS-FLI transcripts we used PCR primer pairs corresponding to EWSR1 and FLI1 exons, up and downstream, of the fusion breakpoint. Using different combinations of primers we compared the number and size of PCR products obtained using cDNA generated from RNA harvested 48 hours post-siRNA transfection of TC32 cells. This analysis showed HNRNPH1 LOF in TC32 cells results in retention of EWSR1 exon 8 when EWS-FLI1 is processed, an event that is predicted to induce an out-of-frame transcript, whereas SF3B1 LOF results in disrupted usage of several exons close to the EWS-FLI1 fusion breakpoint. These findings have been confirmed using the spliceosome inhibitor Pladienolide B (PlaB) that targets SF3B1. PlaB inhibits EWS-FLI1 activity with an IC50 of 2 nM after 24 hours of incubation. 10nM PlaB reduces EWS-FLI1 transcript levels by 90% in just six hours and EWS-FLI1 activity by 50% in less than 12 hours. Importantly, PlaB treatment generates a similar pattern of altered splicing of EWS-FLI1 as SF3B1 silencing. In conclusion, we have identified specific proteins required for the precise splicing of the nascent EWS-FLI1 transcript. Targeting of these proteins has implications for the treatment of ES and may open up strategies for treatment of other cancers driven by fusion oncogenes.

O-12: Multiplexing tumor-specific FISH probes allows single-cell genetic analysis delineating tumor heterogeneity and clonal development in carcinomas and their precursors and in cancer model systems.

Kerstin Heselmeyer-Haddad, Leanora Hernandez, Amanda Bradley, Darawalee Wangsa, Daniel Bronder, Lissa Berroa Garcia, Kathleen Calzone, Pamela Paris, Sonia Andersson, Timo Gaiser, Ailine Stolz, Holger Bastians, Salim Akhter Chowdhury, Russell Schwartz, Alejandro A. SchÃČÂd'ffer, Thomas Ried.

Multiple consecutive hybridizations of multicolor FISH probe panels enable the evaluation of copy numbers of up to 16 different genes within each cell analyzed. This assay can be used in any single cell preparation, like e.g. cytospins of disintegrated FFPE patient material or preparations of cancer cell lines. The selection of genes can be tailored to the tumor type or cancer model investigated. Automated spot counting adapted to this novel assay allows now for the analysis of thousands of cells making it a robust tool to assess tumor clonality and heterogeneity. These single-cell multi-gene copy number data sets can then be used to build phylogenetic tree models using the software FISHtrees (ftp://ftp.ncbi.nlm.nih.gov/pub/FISHtrees) giving new insights into tumorigenesis and progression.3,4 Test statistics computed on the tree model for each sample can be used as features in a machine learning approach to distinguish sample categories (e.g., primary tumors that metastasized vs. primary tumors that did not). We have applied this novel approach to investigate synchronous ductal carcinoma in situ (DCIS) and invasive ductal carcinoma (IDC) of the breast2, non-progressive and progressive prostate carcinomas5, primary cervical tumors and their metastases1 and severe dysplasias (CIN3) and cancers of the uterine cervix, both single and synchronous lesions (manuscript in preparation). We are currently working on a project applying a panel of twelve FISH probes to tumor cell cycle checkpoint and DNA repair defective isogenic colorectal cancer cell lines. Our studies revealed differences between tumors and tumor types in composition and frequency of their major clone populations, clonal development during tumor progression and degree of tumor heterogeneity. E.g., both DCIS and IDC tumors showed generally high intercellular tumor heterogeneity yet conserved signal patterns that are consistent with a nonrandom distribution of signal patterns.2 Synchronous lesions either showed the same major clones in DCIS and IDC or different clones emerged during the progression from DCIS to IDC.2 On the other hand, cervical CIN3 and cancer lesions appear to be less heterogeneous than DCIS and IDC, often revealing dominant clonal populations that can comprise up to 90% of all tumor cells. Of note, so far all of the three synchronous CIN3/cancer cases that we investigated composed of very different major clones, indicating that the respective cancer lesion did not evolve from the CIN3 lesion adjacent to it. The only

common aberration in the adjacent CIN3 and cancer lesions is the gain of TERC, confirming the pivotal role of this genomic change in cervical carcinogenesis. Multiplex-FISH analysis with probes specific for genes that are involved in prostate cancer progression showed that it is possible to distinguish non-progressing prostate disease from progressing cancers with a probe panel consisting of PTEN, MYC and TBL1XR1.5 This will be further validated in larger studies. Phylogenetic tree models showed that cells of prostate lesions that progressed, deviated more from the normal diploid status compared with cells from non-progressing lesions, indicating that the cells in the progressor samples have a trend toward more total chromosomal changes.5 Tree models for primary cervical tumors and their metastases revealed that more than half of the metastatic cells were located in the first two tree levels, while primary tumor cells were more evenly distributed over the first six tree levels.3 Two explanations for this difference could be that 1) clones in the primary tumors had more time to diversify or that 2) stronger purifying selection needs to take place for metastatic clones to evolve, migrate and survive. To accelerate and streamline our data acquisition, we were granted funds from CCR. We recently acquired a spot counting system and developed software with the vendor to allow for automated image acquisition and enumeration of multiplex-FISH hybridization signals. This will enable us to achieve a higher throughput with more cells and genes per sample resulting in more robust and detailed phylogenetic models that will shed more light on clonal development and tumor heterogeneity.

O-13: Outcomes From A Method For Introducing A New Competency Into Nursing Practice (MINC)

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Purpose: Assess outcomes of an intervention to improve registered nurses (RNs) capacity to integrate genomics into practice. Design: Longitudinal study of RNs from 23 MagnetÃĆÂő Designated Hospitals, 21 intervention, 2 controls. Methods: Rogers Diffusion of Innovation guided this study. The intervention: yearlong genomic education, support and supervision program involving administrator and educator dyads. The Genetic/Genomic Nursing Practice Survey assessed nursing workforce attitudes, practices, receptivity, confidence, genomic knowledge and utilization of family history. Results were analyzed using descriptive statistics. Results: Data from 8,151 RNs were analyzed. Response rates ranged from 12-70%, overall 30%. Intervention and control demographics were similar: baccalaureate degrees (61%); staff nurses (74%); experienced (mean 17 years); and spent most time seeing patients (mean 74% intervention, 71% controls). No significant differences between groups were observed in attitudes with most agreeing it was important to become more educated about genomics, nurses have a role counseling patients about genetic risks, and family history should be a key component of nursing care. In the intervention cohort more nurses: believed senior staff saw genetics as an important part of the RN role ($P<0.001$); heard or read about the genomic nursing competencies ($P=0.001$); and had greater intention to learn more about genetics ($P<0.001$). Genomic knowledge deficits and family history utilization did not change significantly. Conclusions: The intervention increased awareness and intention but requires additional intervention to improve knowledge and practice integration. Awareness is the first step in innovation diffusion.

Posters

P-14: Novel near-diploid ovarian cancer cell line derived from a highly aneuploid metastatic ovarian tumor

Anna Roschke, Gina Kim, Jack Zhu, Christopher Lau, Nicole McNeil, Thomas Ried, Paul Meltzer, W. Michael Kuehl, Ester Rozenblum

A new ovarian near-diploid cell line, OVDM1, derived from a highly aneuploid serous ovarian metastatic adenocarcinoma was established and characterized. Metastatic tumor was obtained from a 47 year old Ashkenazi Jewish patient three years after the first surgery removed primary tumor, both ovaries, and the remaining reproductive organs. The cell line was characterized by cell morphology, genotyping, tumorigenic assay, mycoplasma testing, SKY and molecular profiling of the whole genome by aCGH and gene expression microarray. Targeted sequencing of a panel of 197 cancer-related genes was also performed. Hierarchical clustering of gene expression data clearly confirmed the ovarian origin of the cell line. OVDM1 has a near-diploid karyotype with a low-level aneuploidy. A number of single nucleotide variations (SNVs)/mutations were detected in OVDM1, some of them were cancer-related according to COSMIC and HGMD databases. A large number of focal copy number alterations (FCNAs) were also detected, including homozygous deletions (HDs) targeting WWOX and GATA4. Only 10% of copy number alterations overlapped between OVDM1 and the corresponding metastatic tumor samples. In contrast, mutational and gene expression analysis revealed many cancer-related molecular alterations shared by them. Progression of OVDM1 from early to advanced passages was accompanied by acquisition of few additional gross chromosomal rearrangements and more than 100 new FCNAs, both scattered along genome and clustered in a certain regions. Most of newly acquired FCNAs seem to be related to chromotripsis-like rearrangements, indicating the presence of this type of genomic instability in the newly developed cell line OVDM1.

P-15: Molecular Sensitivity Landscape of Rhabdomyosarcoma Reveals Selective BRD4 Inhibition for PAX3-FOXO1 Driven Tumors

Berkley Gryder, Jack Shern, Marielle Yohe, Young Song, Abigail Cleveland, Lesley Mathews-Griner, Xiaohu Zhang, Rajarshi Guha, Paul Shinn, Marc Ferrer, Craig Thomas, and Javed Khan

Background: Rhabdomyosarcoma (RMS) is the most common soft tissue cancer of childhood. Genomic and transcriptomic characterization reveals that this disease is actually composed of two distinct genotypes: those driven by a PAX3 or PAX7 fusion resulting from chromosomal translocation (alveolar histology), and those driven by recurrent mutations in the tyrosine kinase/RAS/PIK3CA axis (embryonal histology). Patients whose tumors harbor a PAX fusion typically relapse, run out of therapeutically beneficial treatment options, and have very poor survival. Approach: To identify small molecules that could potently, safely and selectively inhibit the growth of PAX fusion driven RMS, we assayed a molecular interrogation plate (MIPE) of 1,912 compounds at 11 concentrations each, against 5 cell lines. Two major considerations guided the construction of the MIPE small molecule library: translational potential (1,163 are in clinical studies or approved) and high molecular target diversity. Three rhabdomyosarcoma cell lines were chosen, RH41 (PAX3-FOXO1), RH30 (PAX3-FOXO1 and mutant TP53) and RD (mutant NRAS). Two control cell lines (HEPG2 and CaCo2) were used to de-prioritize broadly toxic compounds. Results: Across all three cell lines, we saw a positive hit rate of 33%, with 630 small molecules showing an AUC $< 80\%$ (corresponding to an average IC50 value of 2 ÃĆÂţM). Roughly 260 of the hits were broadly cytotoxic (such as proteasome inhibitors and antimetabolites). Previously identified sensitivities were confirmed, such as FGFR, IGF1R, and ALK inhibitors and PI3K, AKT and mTOR inhibitors. However, these signaling pathways rapidly develop alternative routes of resistance in vivo due to kinase cross-talk. Regardless of signaling pathway, they all converge in the nucleus, where epigenetic regulation drives gene expression. Interestingly, one of the most selective and potent category of molecules were inhibitors of bromodomain-containing protein 4

(BRD4), an epigenetic reader and transcriptional coactivator. These were found to be selectively active against fusion positive RMS. This trend was confirmed against an expanded panel of 10 total RMS cell lines, IC50 between 270-300 nM for RH41, RH4 and RH5 and an average of 2.0 ÃĆÂţM for RD, CCA, CTR, and RMS559. Gene expression changes upon treatment with BRD4 inhibitor JQ1 was studied by RNA sequencing. Gene set enrichment analysis (GSEA) revealed the inhibition of the PAX3-FOXO1 fusion gene expression signature. Reduction of known downstream targets (MYOD1, MYOG, ALK, MYCN, FGFR4, HDAC5, MEOX1) was accompanied by up-regulation CDKN1A (p21, aberrantly suppressed in RMS to prevent differentiation). Conclusions and Future Directions: A screen identified bromodomain inhibitors as an effective approach to specifically suppress RMS tumor growth. Disrupting transcription-factor/epigenetic coactivator complexes by bromodomain inhibition appears to be a feasible approach for treatment of RMS, especially the PAX3-fusion transcription-factor driven subset, and a highlights new avenue for therapeutic intervention. Candidate PAX3-FOXO1 bound enhancers are also associated with genes selectively down-regulated by JQ1 treatment, suggesting a collaborative role between BRD4 and PAX3-FOXO1 across the aberrantly regulated RMS genome. We are following up these observations with mechanistic studies to interrogate the genome-wide enhancer landscape of RMS and how it is altered upon bromodomain inhibition, using chromatin immunoprecipitation followed by sequencing (ChIP-seq).

P-16: Implementation of next generation sequencing method for clinical testing

C. Christopher Lau, J. Keith Killian, Yuelin Jack Zhu, Marbin Pineda, Robert L. Walker, Yonghong Wang, Ogan Abaan, Sean Davis, Sven Bilke, and Paul S. Meltzer

Adaptation of rapidly maturing next generation sequencing technologies (NGS) for clinical testing is fast becoming requisite for precision medicine owing to its massive advantage in scale, resolution and reproducibility compared to conventional methods. However, implementation of NGS for routine clinical testing presents enormous challenges in critical steps that include specifying the performance characteristics and establishing a quality control system, establishing a sample tracking system, and establishing a result-interpretation and reporting mechanism. Here we describe the process we undertook to establish these critical elements for a 200 cancer related genes NGS-based assay for oncogenomic analysis in accordance to clinical testing standards. To date, over 1000 clinical oncology cases that include blood, saliva, and formalin-fixed paraffin embedded (FFPE) specimens have been analyzed to establish the specifications for performance characteristics that include accuracy, precision, analytical sensitivity and specificity, reference range, and reportable range of test results. A sample tracking mechanism that significantly reduces the chances for patient misidentification, and simultaneously provides independent validation of mutation status was developed and implemented. A mechanism for result interpretation and reporting was also put in place. Through our approach, we have attained >99% concordance with external CLIA-certified laboratories or in-house validation using CLIA-certified conventional sequencing platform for detection of clinically relevant mutations in AKT1, BRAF, EGFR, ERBB2, ERBB4, HRAS, KIT, KRAS, MET, NRAS, PIK3CA, RET, TP53, SDHA, SDHB, SDHC, SDHD and VHL.

P-17: Functional Studies of CtBP in Breast Cancer Using the Lentiviral pINDUCER System

Dae Ik Yi, Jung S. Byun, Tingfen Yan, Natnael Kenea, and Kevin Gardner

C-terminal binding protein 1 and 2 (CtBP1 & 2) are closely related and evolutionarily conserved transcriptional corepressors. CtBP proteins were first identified through the studies of oncogenic adenoviral E1A protein. CtBPs play a significant role in tumorigenesis and tumor progression through various levels of epigenetic regulation. Interacting with transcription factors and chromatin modifying enzymes, CtBPs mediate repression of several tumor suppressor genes and promote epithelial-mesenchymal transition (EMT), resulting in aggressive forms of breast cancer. CtBPs are also considered metabolic sensors in cells due to their ability to bind to NAD+/NADH, the high energy intermediate generated during carbohydrate metabolism. However, little has been known about the precise mechanism of CtBPs in human cancer. In order to elucidate

the mechanism of CtBPs, we are employing an inducible gene expression approach that utilizes the lentiviral pINDUCER system to knockdown and overexpress CtBP genes only in the presence of Doxycycline. With various biomarkers, the system exhibits robust temporal and reversible control of gene expression in a wide range of cell types both in ex vivo and in vivo. The fidelity of this system will enable us to identify, test, and evaluate CtBP pathways that are responsible for more aggressive phenotypic features of cancer, such as EMT, invasion, and metastasis. This system will be employed in a broad array of functional assays at both the molecular and biological level using in vitro and in vivo models in which the levels of CtBP can be inducibly manipulated in wound healing assays, migration and invasion assays, 3D gland formation assays, and orthotopic xenograft mouse models. Together, the functional assays manipulated by the pINDUCER system will shed light on the role of CtBPs in breast tumorigenesis and progression.

P-18: An Evaluation of Three Relative Telomere Length Assays in Dyskeratosis Congenita Patient Samples.

David W. Petersen1, Daniel C. Edelman1, Allison Gomez1, Shahinaz Gadalla2, Philippa Webster3, Lucas Dennis3, Mike Krouse3, Sharon Savage 2, Paul S. Meltzer1

Shortening of telomere length (TL) is recognized as a biomarker in aging, and is increasingly being investigated in a number of cancers. However, methods to measure TL seem to have intrinsic limitations that make them unsuitable for wide-scale acceptance. For example, southern blotting techniques require large amounts (micrograms) of full length genomic DNA and are technically demanding. And while quantitative PCR methods are inexpensive and need only nanogram amounts of DNA, they are very sensitive to slight variations in reagent and sample quality, leading to problems with reproducibility. In our search for the ÃČÂŠbestÃČÂŞ assay we are evaluating a direct hybridization method from NanoStringÃĆÂÍ Technologies. Without the need for target amplification, the NanoStringÃĆÂÍ nCounter System (NS) counts hybridization events of telomeric probes, normalized against 300 endogenous genes. The normalized telomere counts are then compared to a standard human DNA to obtain a relative telomere length (RTL). We first validated the NS assay against cell lines previously characterized with an inÃČÂŘhouse multiplex monochrome quantitative PCR (MMQPCR; Cawthon 2009). This comparison of NS to MMQPCR in 11 cell lines gave a good correlation of $R_{-} = 0.89$. Subsequently, we tested 43 human clinical samples from a dyskeratosis congenita (DC) study that had already had Flow-Fish (FF) TL results. This comparison of all three different assays with clinical samples showed good concordance, encouraging us to continue this evaluation.

P-19: Conservation of aneuploidy-induced gene expression after Trichostatin A treatment

Eric Cardin, Yue Hu, Darawalee Wangsa and Thomas Ried

Epithelial cancers are defined by a specific distribution of chromosomal aneuploidies. The resulting genomic imbalances are tumor specific, are maintained when cells metastasize, and are conserved in cell lines derived from primary tumors. These imbalances result in ploidy-driven transcriptional deregulation marked by low-level expression changes of most genes that reside on chromosomes and chromosome arms that are gained or lost. Correlations between genomic copy number and expression for different tumor entities, including breast, colorectal, pancreatic cancer and derived cell lines have been observed. The strong selection for the maintenance of chromosomal aneuploidies very convincingly suggests a functional relevance. However, it remains unknown to which extent, and how, aneuploidy-dependent transcriptional deregulation contributes to cellular transformation, in particular at early stages of tumorigenesis where these aberrations emerge, and to which extent they are required for the maintenance of the malignant phenotype. Our aim is to address this question by exploring the consequences of disturbing the aneuploidy-induced transcription. The histone deacetylase inhibitor (HDACi) Trichostatin A (TSA) is well known to lead to large scale gene expression changes and have an anticancer effect. Here we assessed if TSA could disrupt the aneuploidy-driven gene expression in the aneuploid colon cancer cell line SW480 and the artificially generated aneuploid cell line DLD1+7. We found that TSA does not disturb the overall aneuploidyÃČÂŘdriven gene expression despite considerable

changes in gene expression but is rather affecting specifically some of these genes. These genes potentially have a role in tumorigenesis and should therefore be studied in more detail. These experiments represent the first attempt to disrupt the aneuploidy-driven gene expression and are important for the understanding of the relationship between aneuploidy and transcriptional deregulation that define the malignant phenotype of epithelial cells.

P-20: Mapping chromosomal interactions: 3C and beyond

Fan Yang, Sven Bilke, Bob Walker, Marbin Pineda, Joshua Waterfall, Jack Zhu and Paul Meltzer

Cancer is most often characterized by gross genomic aberrations, including translocations, amplifications and deletions, as well as somatic mutations. Increasing evidence has shown the importance of spatial organization of chromatin in the nucleus in gene expression and regulation. Potential links between the organization of chromatin and genome stability and carcinogenesis have also been proposed. To better understand the spatial organization of chromatin in breast cancer cells and to explore the potential link between the chromatin organization and gene regulation, we have used chromosome conformation capture (3C)-based techniques, together with chromatin immunoprecipitation and next generation sequencing (ChIP-Seq) to examine the in vivo chromosomal interactions. ChIP-Seq analysis has identified large number of progesterone receptor (PR) and RNA polymerase II (RNAPII) binding sites in T-47D breast cancer cells, which are estrogen receptor (ER)-positive and PR-positive. PR and RNAPII binding sites are detected in promoter proximal regions as well as distal regions, with most located in non-proximal regions. The distribution patterns of PR and RNAPII binding sites suggest that there are potential interactions between promoters and enhancers. 3C analysis on selected PR-regulated genes confirms that there are progesterone-induced chromosomal interactions. We are currently using the Hi-C method to map the genome-wide chromosomal interactions and will correlate the results with ChIP-Seq data to get a better understanding of the gene regulation in breast cancer cells.

P-21: Genome-wide DNA Methylation Profiling in Classic and Variant Hairy Cell Leukemia

Holly Stevenson, Daniel Edelman, Yonghong Wang, Evgeny Arons, Robert Kreitman, and Paul Meltzer

Hairy Cell Leukemia (HCL) is a rare cancer of mature B lymphocytes, comprising 2% of all leukemias. Based on morphology and immunophenotype, the World Health Organization has classified HCL into two distinct diseases, classic HCL and variant (HCLv). Whereas HCL is generally more easily controlled by treatment, HCLv presents as an aggressive disease and responds poorly to current HCL therapies. To better define molecular differences in HCL and HCLv, multiple studies have demonstrated that almost 100% of HCL samples express the oncogenic V600E activating mutation in BRAF, a kinase important in regulating the MAPK/ERK signaling pathway. No BRAF mutations have been identified in HCLv. HCL cells can be further classified into a subgroup based on expression of the immunoglobulin variable heavy chain V4-34 (IGHV4-34). IGHV4-34+/HCL behaves similarly to HCLv, in that it shows poor response to treatment and worse overall survival, expresses wild-type BRAF and harbors mutations in the MAP2K1 gene. In general, the genomes of HCL/HCLv are stable, with the most common chromosomal changes observed as gains in chromosome 5, deletions in chromosome 7q, and deletions in 17p that encompass the TP53 gene. However, data describing epigenetic changes in HCL and HCLv are limited. We thus performed genome-wide DNA methylation profiling on 48 DNAs derived from 28 HCL and 20 HCLv. Data will be presented describing genes and molecular pathways that could potentially be used to classify not only HCL and HCLv, but also those HCL with differential expression in IGHV4. Furthermore, methylation data will be converted and used to characterize any chromosomal aberrations. These results will be useful in identifying further diagnostic criteria and may be important in generating novel treatment strategies for HCL and HCLv.

P-22: Differentially Regulated Genes and Their Correlation with miRNA expression, DNA Methylation, and DNA Copy Variations in paired Tumor/Normal Tissues of TCGA Hepatocellular Carcinoma (HCC) Samples

Hongen Zhang, Paul Meltzer, and Sean Davis

Translational genomics research in cancers, e.g., International Cancer Genome Consortium (ICGC) and The Cancer Genome Atlas (TCGA), has generated large multidimensional datasets from high-throughput technologies. Data analysis at the multidimensional level will greatly benefit clinical applications of genomics information in diagnosis, prognosis and therapeutics of cancers. Here we show the analysis results of gene expression, miRNA expression, DNA methylation, and DNA Copy variations in paired tumor/normal tissue of TCGA HCC samples, aimed at evaluating the relationships between differentially regulated gene expression and other genomics variations. We found that 1817genes are differentially regulated between liver hepatocellular carcinoma and normal tissues (linear model fit, minimum fold change >= 2, and adjusted p value <= 0.05). There are 855 out of 1776 differentially expressed genes are significantly correlated with DNA methylation (adjusted p values < 0.05) and 609 of them have negative correlation coefficients (-0.29 -0.9). Total of 1812 differentially regulated genes have significant negative correlation with one or more miRNA(s) but not every miRNA has significant negative correlation with differentially expressed gene(s). DNA copy number variations are only found in few differentially regulated genes in limited tumor samples. In conclusion, miRNA may play more important roles in differentially regulated gene expression in paired TCGA HCC tumor/normal samples.

P-23: Development of Snakemake Pipelines for Analyzing Next-Generation Sequencing Data

Jack Zhu, Sean Davis, Joshua J. Waterfall, Yonghong Wang, Ogan Abaan„ Chris Lau, Keith Killian, Bob Walker, Marbin Pineda, Hongen Zhang, Sven Bilke, Paul Meltzer

In most bioinformatics analyses, especially for Next-Generation of Sequencing (NGS) data, development of complicated workflows using various tools and generating many files is common. Efficient workflow creation and management can not only ease such development but can also ensure analysis reproducibility. Snakemake is a workflow engine that combines an easy to read text-based definition language with a versatile execution environment that is scalable from single core machines to multi-core servers to clusters. By using Snakemake, we have developed multiple pipelines for analyzing different types of NGS data, including DNA capture sequencing, RNA-Seq, whole genome sequencing, and bisulfite sequencing. Combined with the NIH Biowulf computing environment, Snakemake shortens bioinformatic pipeline development cycles while also enhancing reproducibility and computational efficiency.

P-24: Systematic Identification of Germline Mutations in Neuroblastoma

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Despite improvement of survival using multimodal chemo- and immunotherapy, high mortality and morbidity is still substantial for pediatric patients with metastatic cancers. Recent large-scale sequencing studies of pediatric tumors including neuroblastoma (NB) have been focusing on somatic mutations, and revealed a low somatic mutation rate and surprisingly few recurrently somatic mutated genes in these childhood tumors. Currently, only a small portion of pediatric cancer cases can be explained by somatic driver events; whereas the cause for the majority of these diseases remains unknown. Because of the rarity of neuroblastoma, here we hypothesize that infrequent germline mutations (frequency<0.01 in control populations) may play a role in the initiation of sporadically occurring tumor. To identify rare deleterious germline variants that may

play a role in the initiation of sporadic NB, we utilized whole exome sequencing data from a cohorts of 222 NB (n=222) patients, which is a part of the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) initiative for pediatric cancers. First, high-quality protein-coding changing single nucleotide variants (SNVs) and small indels were called in both paired germline and tumor genomic DNAs. Since NB is an uncommon disease, we excluded common variants with frequency ÃĆÂş1% in any ethnic group in control populations including 1000 Genomes and ESP6500, a non-cancer control population comprising 6503 individuals. Due to our interest in the enriched variants, we further required the frequencies of variants in neuroblastoma patient cohort are higher than those in the ESP6500 dataset. While the indels analysis is still on-going, there are 35596 SNVs fulfilled these selection criteria including known mutations associated with neuroblastoma in genes such as ALK, NF1, PINK1 and BARD1 (10/222). In order to distinguish the disease-causing germ line mutations from rare private variants, we devise an approach combining prior knowledge and statistics which will bin variants in 3 categories: 1) variants in known cancer genes and select human syndromes such as those in the ACMG genes, 2) variants reported in >= 3 tumors from other studies including TCGA and published pediatric cancer sequencing studies, and 3) variants not known to be cancer-associated, but significantly enriched in NB patient cohort comparing to ESP6500. To find the most deleterious germline SNV mutations, we will further filter categories 2 and 3 variants for a C-score >= 20 (top 1%) using Combined Annotation Dependent Depletion (CADD), a method to integrate many diverse bioinformatics predictions (SIFT, PolyPhen, conservation) into a single score. We believe in addition to the notorious cancer-causing mutations our approach will discover novel mutations that are previously unknown to associate with neuroblastoma.

P-25: CTBP-BRCA1 modulate epithelial cell fate in breast cancer

Jung S. Byun, Dae IK Yi, Tingfen Yan, Kevin Gardner

Epithelial cells retain incredible plasticity. They can differentiate and self-renew during embryonic and post natal organ development. An essential development program is the ability to lose their typical epithelial features and change to a mesenchymal state by activating epithelial mesenchymal transition (EMT), a process that is abnormally activated during breast cancer tumor progression and metastasis. BRCA1 is tumor suppressor gene that represses malignant transformation by safeguarding the fidelity of DNA replication and chromosomal segregation and has been suggested to have a possible role in mammary differentiation. C-terminal binding protein (CTBP) is a dimeric nuclear protein and an epigenetic regulator that is activated in the presence of NADH to recruit and target a variety of histone modifying complexes and transcriptional regulators to chromatin, thus providing a defined mechanism through which carbohydrate metabolism can drive epigenetic regulatory events. We have recently shown that CTBP drives epithelial mesenchymal transition (EMT), genome instability, and the acquisition of stem cell tumor-initiating cell-like pathways in breast cancer. In this study we demonstrate that CTBP and BRCA1 show competitive regulation of epithelial feature: BRCA1 depletion perturbs mammary epithelial differentiation, whereas CTBP depletion enhances epithelial differentiation. Genomic profiling reveals that this mode of CTBP/BRCA1 regulation occurs through the regulation of opposing cellular programs and the common target of master regulators of epithelial cell fate.

P-26: Determining the Effect of Histone H3 Mutations on the Epigenetics of Osteosarcoma

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Osteosarcoma is a malignant bone tumor primarily affecting adolescents and young adults. Exome sequencing of osteosarcoma cells lines and tumors has identified G34W and T11A mutations in histone variants H3.3 (H3F3A) and H3.1 (HIST3H1B) in 2% of cases. H3T11 is modified by phosphorylation to promote demethylation of H3K9 and H3G34 mutations most likely affect modification of H3K36, which is methylated in actively transcribed regions. H3G34 mutations are also present in 92% of giant cell tumors of bone and in 15% of pediatric glioblastoma cases. In glioblastoma, mutations in H3F3A cause global changes in histone H3

modification levels and rewrite gene expression. We are currently using the Active Motif H3 PTM Multiplex assay and western blotting to quantify changes in global levels of histone H3 modifications caused by these mutations. We also plan on using chromatin immunoprecipitation combined with quantitative proteomics to identify novel protein partners that may associate with these mutant histone variants in osteosarcoma. As a result of these studies we hope to determine the extent of epigenetic deregulation caused by H3 mutations in osteosarcomas, a disease characterized by genetic instability.

P-27: Oral fluid DNA methylation profiles are determined by leukocyte and epithelial cell composition

Natalia Noyes, Parvathy Retnakumar, M. Scott Killian, William I. Smith, Jr., Yonghong Wang, Sven Bilke, Sean Davis, Lee Helman, Joseph Su, Paul Meltzer

Expectorated human oral fluid (a.k.a. saliva, spit) is a popular, facile, non-invasive specimen for constitutional genome sequencing, epigenomic analyses, epidemiological studies, and nucleic-acid-based detection of pathologic conditions. Despite the prevalence of oral fluid molecular profiling, there are few published reference values for cellular composition in routine expectorated oral fluid DNA extracts; in addition, there is no published reference set for DNA methylation profiling for constitutional epimutations as may be present in oral fluid. Herein we analyze oral fluid Illumina Infinium 450K methylation microarray profiles (GEO platform GPL13534) for cellular composition and proportions, and for methylation levels of several genes subject to epimutation in the human population. Principal component analysis was consistent with a two-lineage mixture of leukocytes and buccal mucosal epithelial cells (BECs), and further showed that leukocytes outnumber desquamated epithelial cells several fold. Additional comparisons incorporating purified lymphoid and myeloid white blood cell subsets revealed that the oral fluid leukocyte component is largely derived from neutrophils (PMN), while lymphocyte-specific epialleles were essentially undetectable by DNA methylation microarray in whole oral fluid DNA extracts. Thus, oral fluid molecular profiling captures the same predominant cell component as bloodÃČÂŚthe neutrophil– while epithelial cells comprise 20%. Differentially methylated targets contributing to oral fluid composition are significantly enriched for enhancers of genes that function in leukocyte and epithelial cell biology. Pediatric versus adult age, but not sex, was found to have a significant correlation with the epithelial/leukocyte ratio in oral fluid. Overall, our results demonstrate that expectorated oral fluid is a biological resource with a definable cell composition and copious DNA yields, and provide reference values for leukocyte and epithelial fractions for molecular profiling studies. Additionally, oral fluid DNA methylation reference databases are useful assets for estimating population frequency of constitutional epialleles/epimutations.

P-28: Noncoding RNAs in the Myc/Pvt1 region: Consequences of the overexpression or suppression of miR-1204 and Pvt1 in developing B cells

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B cell differentiation is a highly ordered process involving changes in the expression of surface markers as well as the onset of rearrangement and expression of heavy and light chain immunoglobulin (Ig) genes. While the cellular progression of B cell progenitors from the bone marrow through to the spleen and on to the periphery is well documented, the precise molecular changes that accompany these transitions still needs more study. It is also recognized that distinct types of B cell malignancies appear to be arrested at specific stages of development. For example, the mouse plasmacytoma represents an end stage lymphoid neoplasm that expresses a rearranged heavy/light chain Ig gene on one allele. On the other allele, there is a translocation that juxtaposes Igh or Igl to a region of murine chromosome 15 that harbors the potential lymphoid target genes of Myc, a noncoding lncRNA Pvt1 and/or a cluster of miRNAs (miR-1204 1208). In another mouse model of B cell neoplasia, splenic marginal B cell lymphoma (SMZL) appears to develop following disruption of the p53 exon 1, resulting in immature small B-lymphocytes that lack the class switch recombination (CSR) or somatic hypermutation (SHM) events of more mature stages. Several highly cited

studies have elegantly shown that accelerated development of B or T lymphomas can be induced in p53-/- mice, but a more recent study has refined this concept by demonstrating that conditional deletion of p53 in other stages of B-lymphoid development can generate different types of B cell malignancy or arrested development. We have recently shown in several human cell lines that binding of p53 can induce expression of the miRNA cluster miR-1204 1208 as well as its host lncRNA Pvt1 and as might be expected, a feedback loop exists in that miR-1204 also increases p53 expression. Given the coincidence that expression of miR-1204 1208 and the host Pvt1 are all found to be up-regulated at the same time in lymphocyte development, when p53 expression is normally turned off (pre-B stage), we have investigated whether constitutive over- or under-expression of Pvt1 or miR-1204 specifically in the pre-B lymphocyte stage induces p53 expression as well as other molecular or phenotypic changes. Lentiviral constructs expressing Pvt1 or miR-1204 under CMV control were introduced into several mouse pre-B cell lines (NFS 112, NFS467, v-preB) spanning the stage when expression of Pvt1 and miR-1204 is detectable. Similarly, CRISPR constructs were designed and introduced to constitutively inhibit the expression of mouse Pvt1 in the pre-B cells. Preliminary results show that over-expression of miR-1204 increases p53 expression in the pre-B cell line NFS467 (with Pvt1 expression) whereas miR-1204 decreases p53 expression in the pre-B cell line NFS112 (with no Pvt1 expression). While experiments with the CRISPR mediated knockout of Pvt1 are still underway, our results already suggest that over- or under-expression of miR-1204 and/or Pvt1 even in slightly different stages of the pre-B cell have dramatically different outcomes on the induction of p53, possibly resulting in different phenotypes as well.

P-29: Pat1 regulates kinetochore structure by protecting centromere-specific histone H3 variant, Cse4 from ubiquitination.

Prashant K. Mishra, Jiasheng Guo, Lauren E. Dittman*, Julian Haase, Elaine Yeh, Kerry Bloom, and Munira A. Basrai

Evolutionarily conserved histone H3 variant Cse4 and its homologs are essential components of specialized centromere (CEN)-specific nucleosomes and serve as an epigenetic mark for CEN identity and propagation. Cse4 is a critical determinant for the structure and function of the kinetochore and is required to ensure faithful chromosome segregation. The kinetochore protein Pat1 regulates the levels and spatial distribution of Cse4 at centromeres. Deletion of PAT1 results in altered structure of CEN chromatin and chromosome segregation errors. In this study, we have shown that Pat1 protects CEN-associated Cse4 from ubiquitination in order to maintain proper structure and function of the kinetochore. PAT1 deletion strains exhibit increased ubiquitination of Cse4 and a faster turnover of Cse4 at kinetochores. Consistent with the role of Psh1, an E3-ubiquitin ligase in ubiquitination of Cse4, transient induction of PSH1 in a wild-type strain resulted in phenotypes similar to a pat1ÃČÂĘ strain including a reduction in CEN-bound Cse4, increased Cse4 ubiquitination, defects in spatial distribution of Cse4 at kinetochores, and altered structure of CEN chromatin. In conclusion, our studies provide novel insights into mechanisms by which Pat1 affects the structure of CEN chromatin and Cse4 levels for faithful chromosome segregation. *Presenting author: Lauren E. Dittman

P-30: Patterns of clonal evolution during cervical carcinogenesis

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In cervical carcinogenesis the gain of the human telomerase gene TERC on chromosome 3q is the decisive genetic event that determines progression of dysplasia to invasive disease. Other genomic imbalances, albeit less frequent, such as gains on 1q, 5p, 8q, and 20q, and losses on 2p, 3p, and 11q are observed as well. To better understand the patterns of clonal evolution during disease progression we developed a multiplex FISH assay with three multicolor probe panels for sequential hybridizations of cervical intraepithelial neoplasia (CIN3) and invasive cervical carcinoma (ICC), including synchronous CIN3/ICC lesions. Our FISH assay targets oncogenes COX2 (1q), TERC (3q), TERT (5p), MYC (8q), and ZNF217 (20q), and tumor suppressor genes ING5 (2p), FHIT (3p), and CHEK1 (11q), and centromere 7 as a ploidy control. Our results confirmed the

dominant role of the gain of 3q: nineteen out of twenty lesions showed a major clonal population with TERC amplification. Nine out of the 20 lesions showed a concomitant loss of the FHIT tumor suppressor gene (3p) suggesting an isochromosome 3q formation, which makes the loss of FHIT the second most frequent clonal event in this group of samples. Other imbalances were present, but, as expected, less common. These results are consistent with the interpretation that the 3q gain provides a growth advantage for cervical epithelial cells. This is supported by our observations that (i) cells with identical patterns of 3q gain are adjacent to each other, hence indicating a clonal proliferation, and (ii) that the only commonality between the major clones in the synchronous CIN3 and cancer lesions was a TERC gain. These findings improve our understanding of clonal development during cervical carcinogenesis and with that our understanding of genome dynamics at early stages of carcinogenesis.

P-31: Cancer evolutionary history of pediatric rhabdomyosarcoma

Li Chen, Jack F. Shern, Jun S. Wei, Marielle E. Yohe, Young K. Song, Laura Hurd, Hongling Liao, Javed Khan.

Background. Rhabdomyosarcoma is the most common soft-tissue sarcoma of childhood typically affecting infants or adolescents. Although there is a growing understanding of the genomic landscape underlying rhabdomyosarcoma, a comprehensive characterization of the dynamics of the mutational process is critical to identifying the driver mutations and thus for the development of more effective diagnostic, prognostic and therapeutic strategies. Next-generation sequencing technologies now provide the ability to survey the entirety of a cancer genome with unprecedented base-pair resolution, and enable the inference of cancer evolution history encrypted in the mutational landscape. Methods. We developed a framework of algorithms to characterize the clonal and subclonal events that define the somatic mutational processes in 44 rhabdomyosarcoma tumors with their matched normal germlines using high coverage (105X) whole-genome sequencing. This technique first estimates the proportion of normal contamination and cancer subclones in a tumor, and corrects for their impact in estimating the timing of genomic variants. Then the variant allele frequency and copy number derived from the sequencing data were used to estimate the timing of genomic variants. The reliability of the method is corroborated by robust estimates of variant timing (coefficient of determination equals to 0.99) and accurate estimates of variant allele fraction (accuracy equals to 0.90), verified by deep sequencing on independent platforms. Results. For PAX-fusion-positive tumors, we detected that 17 out of 19 samples have undergone whole-genome duplication which appears as a major critical event in the middle or late stage development of these tumors. For PAX-fusion-negative tumors, loss of heterozygosity of chr11p.15.5 (usually accompanied with copy gain) is a highly recurrent genomic alteration (24 out of 25 samples) that consistently occurs at early time point of tumor development. Other early events in fusion negative rhabdomyosarcoma include the nonynonymous mutations in FGFR4, KRAS, NRAS and HRAS, and the loss of heterozygosity of chr17. Moreover, In two PAX-fusion-negative tumors without RAS mutations, we identified novel early mutations in thePKN1 gene. Overexpression of one of the mutations in a C2C12 mouse myoblast cell line model demonstrated that the PKN1 mutation inhibited muscle terminal differentiation. Conclusions and future directions. Our results demonstrate two distinct evolutionary paths resulting in PAX-fusion-negative rhabdomyosarcoma (PFN-RMS) and PAX-fusion-positive rhabdomyosarcoma (PFP-RMS). In PFN-RMS, genomic loss of heterozygosity on chr11p15.5 and non-synonymous mutations in RAS pathway (FGFR4, KRAS, NRAS and HRAS), as well as a gene not previously known to be drivers of RMS (including PKN1), occurred early in the evolutionary history of tumor. In contrast, the PAX gene fusion event in fusion-positive RMS tumors is an early detectable event consistently occurring prior to a whole genome duplication event that is commonly observed in these tumors. These findings provide crucial genomic evidence to the growing understanding of the biology of rhabdomyosarcoma and may help identify actionable genetic aberrations for prognosis and targeted therapies. In addition, the potential role of subclonal genomic alterations needs to be determined in a recurrence or metastatic lesion.

P-32: Biomarker investigations in the early development of an anti-angiogenic agent against a novel target CD105

Liang Cao, Yunkai Yu, Andrea Apolo, Austin Duffy, Tim Greten, Fatima Karzai, William Figg, William Dahut

Tumor angiogenesis is a hallmark of cancer development that has been a focus of anti-cancer drug development. Anti-VEGF antibody bevacizumab and many VEGFR inhibitors have been approved to cancer treatment. However, they are often associated with limited improvement on overall survival. Agents against new targets are needed. CD105 or endoglin has been considered as an alternative target for anti-angiogenic therapy due to its expression in proliferating endothelial cells and tumors, and its roles in angiogenesis in human and mouse model. NCI intramural research has taken a significant interest in conducting clinical trials with a chimeric anti-CD105 antibody TRC105 and is responsible for the majority of its phase II development as a single agent. Here we report biomarker results of three trials in advanced urothelial, hepatocellular, and castration resistant prostate cancer. In the case of prostate cancer, the data indicates a dose-dependent clearance of target antigen (CD105) from circulating plasma. There is an evidence of anti-angiogenic activity for TRC105 that is specifically associated with the clearance of plasma CD105. Additional data will be presented on early stage exploratory studies examining the association of antigen clearance, VEGF induction, and some clinical endpoints, including objective response and changes in PSA.

P-33: Technical Difficulties in Quantifying NGS Libraries for Assaying on Illumina Sequencing Platforms

Marbin A. Pineda, Robert L. Walker, Jennifer Walling, C. Christopher Lau, J. Keith Killian, Mark Mackiewicz, Paul S. Meltzer

Loading Next Generation Sequencing libraries onto the various Illumina platforms is the simplest but most perplexing issues faced by many sequencing facilities. Incorrectly loading a sample onto a sequencer can yield low data or worse no data, driving up cost and turn-around time of running experiments and/or clinical assays. This can primarily be attributed to the random distribution of binding oligos on the Illumina flowcell. Not having an ordered flowcell introduces operator loading inconsistencies. Accepted standard practice is using qPCR as a means for quantifying libraries, however qPCR methods lack the ability to accurately represent a direct measurement of molarity in a diverse population of molecules within a given library. Libraries not having a uniform size distribution or varying degrees of quality need better methods for quantification. Here, we will outline various obstacles and some new methods in the field to overcome the sample loading challenge. We believe a more robust and standardized method for library quantification, can reduce the cost of experiments by achieving the highest possible output from each run of our sequencers

P-34: CtBP Drives Phenotypic Changes in Breast Cancer Cells in Response to Altered Metabolism

Natnael Kenea, Dr. Jung S. Byun and Dr. Kevin Gardner

Breast Cancer is one of the major public health problems. Annually, close to 40,000 people die of breast cancer in the USA and close to 500,000 people worldwide. There are various risk factors associated with breast cancer like age, race and diet.Obesity is associated with increased risk of breast cancer. C-terminal Binding Protein (CtBP) is a nuclear protein that has a binding site for Nicotinamide adenine dinucleotide (NADH). NADH has been found to be responsible for stabilizing the dimers of CtBP (CtBP1 and CtBP2). NADH is a byproduct of Carbohydrate metabolism. Thus, CtBP is influenced by diet. CtBP has been linked to tumorigenesis and tumor progression. It promotes EMT (Epithelial-Mesenchymal Transition) by inhibiting E- cadherin (Epithelial Marker) and other proteins important in epithelial differentiation. In these presentation an the results CtBP over expression and varying level of glucose on cell migration in MCF-7 cell

line using wound healing assay is presented. Also, the comprasion of E-cadherin and Vimentin staining with CtBP staining in tissue section using Immunohistochemistry (IHC) is discussed.

P-35: Establishment of Human Rectal Tumor Cell Lines with ROCK Inhibitor and Feeder Layer Cells

Nicole McNeil, Ph.D., Alison McBride, Ph.D, Bruce Brenner,M.D. Zhongqui John Zang,M.D. Ph.D., Richard Schlegel, M.D. Ph.D., Daniel Rosenberg, Ph.D. and Thomas Ried, M.D.

Adult human tissue has been difficult to passage in culture as it is limited by the early onset of senescence which causes finite cell proliferation. The conditional reprogramming of epithelial cells in combination with irradiated mouse fibroblast feeder cells and a Rho kinase (ROCK) inhibitor Y-27632 offers a unique method to propagate primary adult cells in vitro. The combination of ROCK and feeder layers convert the epithelial cells to a proliferative state by altering cell growth without the use of exogenous viral or cellular gene expression (Schlegel R et al, 2012). Our lab is involved in a prospective study to identify the biological basis of treatment response in patients with rectal cancer. While surgery is the current mainstay in the treatment of rectal cancer, not all patients respond equally well, with a range from complete response to continued tumor growth. Therefore, the goal of this project is to identify biological or clinical markers that can be utilized prior to initiating treatment that can predict which patients will respond well to neoadjuvant regimens or those who have tumors resistant to chemotherapeutic agents and will benefit from surgery. We are in active collaboration with scientists from the University of Connecticut (Dr. Daniel Rosenberg), Georgetown University (Dr. Richard Schlegel and Dr. Bassem Haddad) , NIAID (Dr. Alison McBride) and NCI (Dr. Stephen Chanock and Jim Doroshow) who contribute expertise, specimen and protocols for tissue culture using Rho kinase inhibitors and haplotype analysis. In our study human rectal tumor and matched normal biopsies are grown on irradiated Swiss 3T3 fibroblast cells with Y-27632 treated media to establish cell lines. To date we have established six cell lines. Array CGH has identified patterns of chromosomal imbalances in these cell lines such as gains of chromosomes 7, 13, and 20 as well as loss of chromosome 18 that are typically observed in rectal cancer. Gene amplifications of MYC as well as EGFR have been observed in some of the cell lines. Future directions are to expose cell lines to radiation and 5-FU in order to compare sensitivity with clinical response; to generate expression profiles after exposure to radiation and 5-FU; to validate expression profiles as biomarkers for response prediction in independent data sets; and to develop proteomic fingerprint of rectal carcinoma from blood and tumors from patients with rectal carcinoma.

P-36: Large Construct Capture (LCC): An economical, quick, customizable method to target regions of interest for Next Generation Sequencing (NGS).

Robert L. Walker, Joshua J. Waterfall, Holly S. Stevenson, Ogan D. Abaan, Marbin A. Pineda, Yuelin J. Zhu, Wei-Dong Chen, Walter M. Kuehl, Paul S. Meltzer.

To investigate rearrangements and deletions in specific regions of interest in cancer samples, a selective reagent is required which enriches for the desired sequences while diminishing representation from outside the targeted region. By reducing sample complexity to a more narrow focus, the volume of information that NGS provides can be allocated across a more highly multiplexed sample pool to a greater depth. Custom selective reagents are commercially available in the form of RNA or DNA baits from a number of companies (eg. Illumina, Agilent and Roche), but the cost of these reagents may be prohibitively expensive, and turnaround time between design and implementation can be slower than desirable. Here we describe a cost effective method using Fosmids or BACs to prepare capture baits tiling highly definable, megabase-sized regions. Preparation can be quickly accomplished in any molecular biology laboratory using conventional skills and reagents.

P-37: The optimization and execution of RNAi screens in multiple ovarian cancer cell lines to identify individual and combinatorial molecular targets for the treatment of ovarian cancer

Sirisha Chaka, Marianne Kim, Christina Annunziata, Natasha Caplen

Ovarian cancer is the most lethal gynecologic cancer in USA. Many efforts, including gene expression profiling, SNP analysis, comparative genomic hybridization, and exome sequencing have revealed that ovarian cancer is extremely heterogeneous. This implies that therapeutic strategy should be designed based on molecular characteristics and may require the simultaneous targeting of multiple molecular targets. Previous shRNA RNAi screens, conducted by our collaborators, identified 55 genes as essential for the survival of multiple ovarian cancer cell lines. In order to validate and prioritize these 55 candidate genes for further study siRNA screens were conducted in six ovarian cancer cell lines; Ovcar3, Igrov1, Ovcar5, A2780, Skov3, and Ovcar8. A second screening study investigated if any of the 55 genes, when silenced, enhances the sensitivity of ovarian cancer cells to a new inhibitor of the checkpoint kinase 1 (CHK1) LY2606368 (Eli Lilly). To conduct these screens we developed a standard experimental workflow for each cell line. This included optimization of the number of cells needed per well and the concentration of the transfection reagent required for high efficiency transfection, as well as determination of the time at which the end point assay (cell viability) should be performed. For the drug screen we also determined the concentrations of LY2606368 required to identify sensitizing targets. Parameters were tested initially using a manual set up in 384 well plates. Once standard conditions had been developed these steps were executed using appropriate automated liquid handling devices. For RNAi screening, two siRNAs per gene (Qiagen) were tested in 384-well format and to assess cell viability we used an assay of ATP metabolism (CellTiter-Glo, Promega), The quality control parameters for these screens included confirmation of transfection using a positive control siRNA that induces cell death and an established sensitizing target of the CHK1 inhibitor, IKBKE. Based on an assay of cell viability (CellTiter-Glo, Promega), nine genes, EPHB1, FER, MAP3K7, MAP3K8, MGC42105 (NIM1), PRKCA, PLK1, ERBB2, and WEE1 were confirmed as essential for the growth of four ovarian cancer cell lines, while six genes, EPHB1, FER, MAP3K7, PLK1, ERBB2, and WEE1, were identified as essential for the growth of all six ovarian cancer cell lines. The silencing of MAP3K7 also sensitized several of the ovarian cancer cell lines to the CHK1 inhibitor LY2606368. Other genes, that when silenced, sensitize multiple ovarian cancer cell lines to the CHK1 inhibitor included NLK and BRD4. Follow up studies, to be conducted by our collaborators, will include the use of small compound or peptides to phenocopy the effects of inhibiting the function of candidate proteins identified by RNAi screening.

P-38: Development of anti-FGFR4 monoclonal antibodies as therapeutic agents for human rhabdomyosarcoma

Baskar S., Ovanesian M., Labitigan1 R.L.D., Zhu Z., Dimitrov D.S. and Khan J.

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma of childhood with two major subtypes embryonal (ERMS) and alveolar (ARMS), and current treatment modalities have yielded event free 5-year survival in only 30% of the patients with high-risk disease. Therefore, there is an absolute need for novel strategies and to identify and validate clinically relevant targets for the treatment of RMS. The fibroblast growth factor receptor 4 (FGFR4) is a very attractive therapeutic target because: 1) the FGFR4 gene is over expressed in RMS, 2) it is crucial for survival, proliferation, metastasis and drug resistance, 3) activating mutations in the kinase domain lead to aggressive growth and poor survival in patients with alveolar RMS and 4) genetic or pharmacologic inhibition of FGFR4 signaling inhibited tumor growth in vitro and in vivo. Monoclonal antibodies (mAbs) against specific antigens expressed on cancer cell surface have gained importance as potential therapeutic agents that may be used either alone or in combination with chemotherapeutic drugs. We have developed several mAbs against human FGFR4 protein from rabbit and mouse by hybridoma technology, and from human immunoglobulin libraries by recombinant DNA technology. Here, we demonstrate the ability of these mAbs to bind the native FGFR4 molecule, by flowcytometry, on RMS cell lines of both subtypes, and dose response curves exhibited higher binding in ARMS cells than ERMS cells.

More importantly, significant binding was also noticed in freshly isolated tumor cells from a breast metastatic nodule of a patient with ARMS. The binding of anti-FGFR4 mAbs to transfected cell line expressing FGFR4, but not the vector control (FGFR4 negative) cell line, indicated the specificity of the reaction. Many normal human tissues do not express FGFR4 with exception of liver and lung that show significantly lower expression of this gene. However, we found that primary human hepatocytes do not express FGFR4 protein on the cell surface. These observations suggest that FGFR4-targeting therapeutics may be safe for human treatment. Furthermore, cell surface FGFR4 can mediate internalization of bound mAbs and therefore, anti-FGFR4 mAbs can be used as a vehicle to deliver a cytotoxic payload in the form of small molecule drugs and toxins. Ongoing investigations are aimed at evaluating the full potential of anti-FGFR4 mAbs, their derivatives including antibody drug conjugates and immunotoxins as potential therapeutic agents for the treatment of patients with RMS. This study is supported in part by the intramural research program of the National Cancer Institute, National Institutes of Health, grants from St. BaldrickÃČÂŢs Foundation (to SB) and Stand Up To Cancer ÃČÂŘ St. BaldrickÃČÂŢs Pediatric Dream Team Translational Cancer Research Grant, Grant Number SU2C-AACR-DT11-13.

P-39: What is in control of the 3D topology of the genome?

Sven BIlke, Paul S. Meltzer

The linear sequence of DNA is only the most basic description of the genome. It is increasingly understood that the three dimensional (3D) nuclear topology is functionally important [1]. It seems plausible that a change in DNA topology during cell differentiation or disease progression is a key component of cell fate determination as it alters the proximity of trans-regulatory elements. In a recent study , Dekker and co-workers introduced a novel method, HiC, allowing for an unbiased genome wide study of 3d conformations producing a probability map of DNA-DNA contacts in an ensemble of cells [2]. Here we aim to identify genomic parameters correlating with the 3d-structure measured in [2]. We have developed an initial model based exclusively on DNA sequence related observables and a set of mixing parameters. Using Monte Carlo optimization techniques, we identify two major sequence features contributing to the contact matrix. The resulting model reproduces the empirical consensus contact probability map described in [2] with Pearson's correlation $r > 0.71$. For comparison, intra-experiment correlation of the data in [2] ranges from 0.55 to 0.89. [1] G. Cavalli and T. Misteli. Functional implications of genome topology. Nature Structural & Molecular Biology, 20(3):2909, Mar. 2013. [2] Lieberman-Aiden,E., Dekker,J. et al, Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science, 326(5950) 289-293 (2009).

P-40: Analysis of JAZF1 loss-of-function reveals its regulation of prostate cancer-associated genes

Tamara Jones, Helen Gharwan, Wei Tang, Suntae Kim, Stefan Ambs, Ludmila Prokunina-Olsson, Natasha Caplen

Genome-wide association studies have mapped susceptibility to prostate cancer to a sequence variant within intron 2 of the gene that encodes a putative transcriptional co-factor JAZF1. In order to translate GWAS findings into clinically relevant information that will ultimately enable us to improve the management of prostate cancer it is essential to identify the biological role susceptibility variants play in cells and tissues. To date, only a few studies have addressed the biological function of JAZF1 and none in prostate cancer. To investigate the function of JAZF1 we generated expression profiles of prostate cancer cell lines, in which JAZF1 was silenced. RNA-Seq was conducted on two metastatic prostate cancer cell lines, LNCaP and DU145, in which siRNA-mediated RNAi had been employed to induce JAZF1 loss-of-function (LOF). For this study, three different JAZF1 siRNAs were used, along with a siRNA (siNeg) serving as control for transfection-related changes in gene expression. Approximately 1,500 genes were identified as deregulated in both cell lines. Gene ontology analysis of this 1,500 genes showed enrichment for genes associated with prostate cancer (p=2.4x10-06, IPA, Ingenuity Systems). To select genes for follow up analysis we considered three criteria, (1) the consistency and degree of change in gene expression observed following

JAZF1 silencing, (2) evidence in the literature of a candidate gene functioning in prostate tumorigenesis, and (3) indication of a correlation of the expression of a candidate gene with the expression of JAZF1 in prostate cancer samples. To obtain evidence of a correlation of the expression of a candidate gene and JAZF1 expression in prostate cancer samples we examined multiple publically available large-scale data sets through the cBioPortal (http://www.cbioportal.org/public-portal/). Twenty-eight genes were selected for initial follow-up, including 11 genes down-regulated following JAZF1 LOF: AURKA, CDKN2C, E2F1, ID1, ID3, MED30, MEIS1, MEN1, MST1R, TMEM245, and TP53, and 17 genes up-regulated following silencing of JAZF1: CDKN1A, CDKN1C, CLU, DDX3Y, DNMT3B, FAM217B, GFPT2, JAG1, MAK, MAP2K4, MET, NRAS, PRKCE, RHOB, RNASEL, SH3PXD2A, and TP53INP1. Using Nanostring multiplex expression profiling we confirmed, at 48 and 72 hours post-siRNA transfection, the deregulated expression of 26 of these 28 genes following silencing of JAZF1. The three genes showing the greatest down-regulation following JAZF1 LOF were MEIS1, ID1, and AURKA; the top three genes showing the greatest up-regulation following JAZF1 LOF were CDKN1C, MET, and SH3PXD2A (TKS5). We are currently investigating whether the changes in the expression of these six genes following JAZF1 LOF results in changes in their protein expression and alters cellular processes associated with the function of these genes.

P-41: Investigating the Mechanisms of p53 Loss in the Highly Unstable Osteosarcoma Genome

Tianyi Wu, So Young Moon, Sven Bilke, Robert L. Walker, Marbin Pineda, Hongen Zhang, Sean R. Davis, Yuelin Zhu, Ashok R. Venkitaraman, Paul S. Meltzer

Osteosarcoma is the most common primary bone tumor in the first two decades of life. Genetically, it is notable for its genomic heterogeneity and complexity with many observed structural rearrangements and copy number variations. However, as with the majority of cancer types, loss of p53 appears to be an important milestone in osteosarcoma tumorigenesis and is almost universally present in osteosarcoma tumors. Unusual among cancers, disruption of the TP53 locus in osteosarcoma occurs frequently through structural rearrangements instead of the more commonly observed point mutations. We propose that genome instability driven by replication stress and DNA misrepair plays a critical role in the disruption of TP53 in osteosarcoma. We will develop inducible human cell line models of TP53 rearrangements that we will then use to interrogate DNA damage and repair pathways to uncover cell-type or lineage specific vulnerabilities leading to structural alterations particularly at the TP53 locus. Loss of p53 allows progressive accumulation of genome alterations. Elucidating the mechanisms underlying genome instability and p53 loss in osteosarcoma and its precursor lesion will lead to new insights and development of novel detection and therapeutic strategies.

P-42: Genome-wide Co-assembly Analysis of BRCA1 and CtBP at Sites of Active Transcription

Tingfen Yan, Jung Byun, Dae Ik Yi, Natnael Kenea, Songjoon Baek, Kevin Gardner

The C-terminal binding protein (CtBP) is a transcriptional co-repressor. It assembles the factors regulating chromatin structures or recruits other repressors to the promoter regions of the target genes. We are interested in investigating the relationship of the genomic binding-site occupancy of CtBP with its co-repressor or transcription factors using ChIP-seq data. Heatmaps of ChIP-seq tag density, General Linear Models (GLM) and clustering were utilized. MCF7 ChIP-seq data including CtBP and BRCA1 from our lab, and p300, DNase I, Pol II, H3K4me3, H3K27ac, H3K27me3, H3K9me3 from NCBI SRA were applied for this study. The heatmaps of tag density showed CtBP, BRCA1, p300, Pol II, co-occupancy and DNase I hypersensitivity at the transcription start site (TSS). The binding sites of H3K4me3 and H3K27ac bracketed the TSS; and occupancy by H3K27me3 and H3K9me3 was excluded from the TSS with no other clear trend. Correlation analysis indicated that CtBP occupancy was highly correlated with BRCA1 throughout the genome, a moderate correlation with p300, DNase I, Pol II, H3K4me3 and H3K27ac and the lowest correlation with H3K9me3 and H3K27me3. The GLM results identify a genomic fingerprint that accurately predicts CtBP binding sites and provides potential evidence that CtBP may compete with BRCA1 at the genomic binding sites.

These results suggest that CtBP and BRCA1 participate in dynamic interaction at a common set of genomic binding sites. The functional consequences of these interactions are currently under investigation.

P-43: Dbf4-Dependent Kinase regulates cellular levels of Cse4 to prevent Cse4 mis-localization to non-centromeric chromatin in S. cerevisiae

Valerie E. Garcia, Lars Boeckmann, Wei-Chun Au, Michael Costanzo, Charlie Boone, Munira A. Basrai

The evolutionarily conserved Dbf4-Dependent Kinase (DDK) is a complex consisting of Cdc7 and Dbf4. DDK is a replication factor that has recently been shown to localize to centromeres. In a genome wide screen in Saccharomyces cerevisiae we found that mutants of DDK show synthetic dosage lethality (SDL) with excess Cse4, the centromeric histone H3 variant. Growth assays were used to confirm the SDL of cdc7 and dbf4 strains with excess Cse4. These results prompted us to explore the underlying mechanisms causing this SDL. First, we performed a ChIP experiment to test if excess Cse4 titrates DDK away from centromeres. Our results showed that excess Cse4 does not inhibit the association of DDK with centromeres. Motivated by results observed for an E3 ubiquitin ligase mutant, psh1, that also exhibit SDL with excess Cse4, we next investigated if the SDL phenotype may be due to altered biochemical properties and mis-localization of Cse4 in the DDK mutants. Increased protein stability, mis-localization to non-centromeric regions, and enrichment of Cse4 in the chromatin fraction have been previously described to contribute to the SDL of psh1 mutant with excess Cse4. Mis-localization of Cse4/Cid/CENPA to non-centromeric regions has been shown to cause chromosome segregation defects and contributes to aneuploidy in yeast, flies, and humans. Similar to the psh1 mutant, we also observed enrichment of Cse4 in the chromatin fraction of a dbf4 strain compared to a wild type control. This observation was confirmed by an immunofluorescence approach using chromosome spreads, which showed that excess Cse4 is mis-localized to non-centromeric regions in a cdc7 strain. Assessment of Cse4 protein stability in cells treated with cycloheximide showed that Cse4 is more stable in cdc7 and dbf4 mutants. Analysis of ubiquitination patterns showed reduced levels of ubiquitinated Cse4 in a cdc7 mutant, which is consistent with the increased stability of Cse4. Taken together, our results provide evidence for a new role of DDK in regulating cellular levels of Cse4 in order to prevent Cse4 mis-localization to non-centromeric chromatin.

P-44: Novel role of the essential SCF Met30 E3 ubiquitin ligase complex in regulating proteolysis and localization of Cse4 to maintain faithful chromosome segregation.

Wei-Chun Au, Anthony Dawson, Peter Kaiser, Charlie Boone, Richard Baker, Michael Costanzo, Chad Myers, Anastasia Baryshnikova and Munira Basrai

Evolutionarily conserved centromeric histone H3 variant, CENPA is an epigenetic mark for centromere identity, essential for kinetochore assembly and faithful chromosome segregation. Importantly, overexpression and mislocalization of CENPA are observed in as many as 23% of cancers. Using budding yeast as a model system, we were able to establish a causative link between misregulation of CENPA (Cse4 in yeast) expression and localization and chromosome segregation errors. Previous studies have shown that centromere-specific localization of Cse4 is regulated by ubiquitin-dependent proteasome pathways. Both the N- and C-termini of Cse4 play critical and distinct roles in preventing the mis-localization of Cse4 to non-centromeric regions. It has been shown that the C terminus of Cse4 is targeted for ubiquitylation by the E3 ligase, Psh1. Given the fact that Cse4 can still be ubiquitinated and degraded in a PSH1 null strain, we hypothesized that additional pathways/mechanisms for Cse4 proteolysis must regulate its cellular levels and prevent it from being mis-localized. . To gain comprehensive insights into mechanisms that regulate Cse4 localization and protein levels when it is overexpressed, we undertook a Synthetic Genetic Array (SGA) screen to interrogate the entire genome for genes whose deletion or mutations exhibit synthetic growth defect only when Cse4 is overexpressed. The SGA screen identified multiple members of a complex and also PSH1 thereby validating the approach we used. We pursued further studies to investigate the role of the essential SCF Met30 E3

ubiquitin ligase complex in regulating ubiquitin-mediated proteolysis of Cse4 to prevent its mis-localization.. Growth assays confirmed the results of the SGA screen as mutants of the SCF Met30 complex (Met30, Skp1, Cdc34 and Cdc53) exhibited synthetic growth defects upon Cse4 overexpression. Mutants for SCF (met30-6 and cdc34) show increased stability of Cse4 that is further correlated with defects in ubiquitination and an enrichment of Cse4 in the chromatin fraction. Co-immunoprecipitation combined with the results of in vitro assays demonstrate that SCF complex ubiquitinates Cse4 via a direct interaction with the F box protein, Met30. Consistent with proteolysis as a mechanism to exclude Cse4 from non-centromeric regions to preserve genome stability, we observed that met30-6 strain exhibits defects in chromosome segregation. Taken together, we conclude that SCF Met30 E3 complex regulates ubiquitin mediated proteolysis of Cse4 to prevent its mis-localization to non-centromeric regions for faithful chromosome segregation.

P-45: 4C-seq to identify MYC promoter interactions in myeloma cell lines with different MYC locus rearrangements

Wei-Dong Chen, Joshua J. Waterfall, Robert L. Walker, Paul S. Meltzer, W. Michael Kuehl

MYC locus rearrangements have been identified in 50% of multiple myeloma (MM) tumors and 85% of MM cell lines (MMCL). There are 3 categories of rearrangements: 75% involve a super enhancer, 10% a possible conventional enhancer, and 15% no obvious enhancer. We have used 4C-seq technology with the goal of identifying novel MYC promoter interactions that are focused on the latter 2 categories. Initially we used two MMCL: 1) Karpas 620 has a complex translocation that repositions MYC within 500 kb of a 3ÃČÂŢ IGH super enhancer, providing a positive control; and 2) Kp6 has a 90 kb tandem duplication located about 500 kb telomeric to MYC, in a region that has a CTCF binding site that is flanked by H3K27 marks. In addition to providing details about different protocols used to make 4C.seq libraries, data showing the interactions of the MYC promoter with distal sequences will be shown for both Karpas 620 and Kp6.

P-46: Developing Precision Therapy Protocols: A Pilot Study for Children and Young Adults with Relapsed or Refractory Cancers

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Objectives. Our goal was to use a combination of whole exome sequencing (WES) of tumor/normal DNA and whole transcriptome sequencing (WTS) for children and young adults with relapsed or refractory cancers to identify actionable targets. We tested our theoretical model for precision therapy, and here we report the results of our feasibility study of the proposed clinical model that has been initiated but not fully implemented at the National Cancer Institute as well as at other medical centers around the United States. Methods. WES was performed on matched tumor and normal samples from pediatric oncology patients with non-central nervous system solid tumors to identify germ line and somatic mutations. In addition, WTS was performed on all tumor samples for fusion genes, gene expression profiling, and expressed variants. Actionable changes were defined as 1) reportable incidental findings in the germ line, 2) change in diagnosis, 3) somatic changes that can be targeted by drugs that are FDA approved or undergoing clinical trials. Results. As of May 2014, over 225 patients and family members were enrolled in our pilot study. Of these patients, 48 had pediatric solid tumors outside of the central nervous system (CNS), and with matched tumor/normal DNA and RNA available for analysis, with some patients having multiple biopsies from different time points or metastases. Using exome data only, from our 48 samples of relapsed or refractory pediatric solid tumors, we found a median of 8 somatic mutations in exome sequencing, a higher mutational burden compared to primary pediatric malignancies previously reported in literature. Utilizing transcriptome data further refined our somatic mutation calls to a median of 1 expressed somatic mutations per sample. We also found that it was moderately helpful to sequence relapsed tumors at multiple time points, as driver mutations seen in initial relapse samples had a continued presence in biopsies at later time points, but new additional mutations were noted after interim chemotherapy had been applied. Seven of the 48 patients (14.5%) contained a mutation

that we considered to be ÃČÂŠtargetableÃČÂŞ in a clinical trial setting. These include BRAF, GNAQ, PIK3CA, ALK, and STAG2. No patients had multiple targetable mutations. The majority of patients had mutations that were likely to be oncogenic. Many of these mutations were previously reported but were novel in the tumor type. As a result of sequencing their tumors, 2 patients (4%), had a change in their tumor diagnosis because of the presence or absence of diagnostic fusion gene markers. Two novel gene fusions were also identified in this cohort, CIC-FOXO4 and BRAF-ATG4C. In the germ line sequencing data of 5 of our patients (10%), we discovered deleterious mutations in genes published by the American College of Medical Genetics, which are reportable incidental findings. In sum, a total of 13 patients (27%) had actionable mutations that were detected by this genomic approach. Conclusions. Our pilot study shows the clinical feasibility of next generation sequencing in a diverse population of relapsed and refractory pediatric solid tumors excluding CNS diseases. We discovered targetable pathways that are present in tumors, prompting clinicians to consider treatment that would otherwise not be prescribed. Using our clinical sequencing protocol with analysis pipelines that were constructed during our pilot study implementation, our goal is to be able to provide results within 2 weeks from the date of biopsy. In a relapsed setting, it is critical to know the molecular targets in these relapsed tumors, as these patients have a 5-year survival rate of only 4-35%, depending on the specific diagnosis. Further implementation of the techniques used in this pilot study has the potential to change the future practice of the developing field of personalized medicine.

P-47: LncRNA-PHLDA3, a p53-regulated long non-coding RNA, regulates cell viability following DNA damage

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The p53 tumor suppressor is a sequence-specific transcription factor required for the cellular response to stress conditions such as DNA damage. For example, p53 regulates the expression of genes involved in cell cycle arrest, apoptotic cell death, and senescence. Long non-coding RNAs (lncRNAs) are a class of RNA molecules of more than 200 nucleotides that are non-protein encoding, but function by regulating the expression of other genes through diverse mechanisms, including epigenetic and transcriptional mechanisms. To date, very few lncRNAs have been functionally characterized. In this study, we aimed to identify p53-regulated lncRNAs and to determine the function of these lncRNAs in the cellular response to DNA damage. To identify p53-regulated lncRNAs we used cDNA microarray analysis of RNA harvested from three different colorectal cancer cell lines, HCT116, RKO, and SW48, following treatment with the p53 activator Nutlin and vehicle matched control samples. Among several lncRNAs we identified as activated by p53, was lncRNA-PHLDA3. The gene encoding lncRNA-PHLDA3 is located on chromosome 1, distal of PHLDA3, an established p53-regulated protein-coding gene involved in cell proliferation and apoptosis. We confirmed that lncRNA-PHLDA3 is upregulated by p53-activation mediated by different cellular stress conditions including, DNA damage induced by doxorubicin or camptothecin (CPT), and ribosomal stress induced by actinomycin D (ActD). The lncRNA-PHLDA3 gene is comprised of two exons and encodes a transcript of 598 nucleotides in length. Using RNA purified from cytoplasmic and nuclear fractions we determined that lncRNA-PHLDA3 is predominately localized in the cytoplasm. Using siRNA gene silencing approaches we showed that depletion of lncRNA-PHLDA3 enhances CPT cytotoxicity. Control siRNA-transfected HCT-116 cells showed an approximate 40% reduction in cell viability when treated with 50 nM CPT for two days, whereas lncRNA-PHLDA3 silenced HCT-116 cells showed about a 80% reduction in cell viability when treated with 50 nM CPT. Furthermore, cell cycle analysis by FACS of CPT treated HCT116 cells found, that in the absence of lncRNA-PHLDA3, cells failed to retain a functional G2/M arrest state, leading to apoptotic cell death. Similar results were obtained following the induction of ribosomal stress by ActD treatment. Importantly, using p53 isogenic knockout HCT116 cells we determined that the effect of silencing lncRNA-PHLDA3 expression on cell viability and apoptotic cell death is p53-dependent. In conclusion, we have identified a new lncRNA target of p53, lncRNA-PHLDA3 that functions in regulating cell survival and apoptosis in response to cellular stress.

P-48: Syngeneic and allogeneic hematopoietic stem cell transplantation in mice with myelodysplastic syndrome

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The myelodysplastic syndromes (MDS) are clonal hematopoietic malignancies characterized by dysplasia, ineffective hematopoiesis and a propensity for progression to acute myeloid leukemia (AML). Allogeneic hematopoietic stem cell transplantation (HSCT) remains the only curative therapy for the majority of patients. However, overall survival (OS) of patients with MDS following allogeneic HSCT is only about 40%, due to both relapse and non-relapse mortality (NRM) including graft versus host disease (GVHD). Available data suggests that long-term survival following HSCT for MDS is due both to myeloablative therapy and a graft versus tumor (GVT) effect. Mice that express a NUP98-HOXD13 (NHD13) transgene develop MDS with virtually 100% penetrance. In order to develop a pre-clinical model for the study of MDS HSCT, we transplanted NHD13 mice, which are bred on a C57 Bl6 background, with bone marrow harvested from syngeneic C57Bl6 donors. Sub lethally irradiated (650 rad) recipient NHD13 mice transplanted with syngeneic donor cells relapsed early, with no therapeutic benefit in terms of hematologic parameters in peripheral blood or survival. However, lethally irradiated (1000 rad) recipient mice that were transplanted with syngeneic donor bone marrow (BM) showed complete normalization of peripheral blood counts significantly enhanced survival (median survival of 15 months) compared with non-transplanted NHD13 mice (median survival 10 months). Although there were no detectable MDS cells for up to 38 weeks post-transplant, all mice eventually relapsed and died. In order to determine if a GVT effect could enhance survival, we performed 3 types of allogeneic HSCT with donor BM that was mismatched at minor histocompatibility antigen loci (C3H.SW x C57Bl6 donors); donor BM only, donor BM with donor splenocytes (6 x 10E06 CD3+ T cells), and donor BM with donor regulatory T cells (Treg). None of these forms of allogeneic HSCT let to enhanced survival compared to that achieved with syngeneic HSCT. The early relapse rate for allogeneic HSCT with donor BM only was decreased compared to the syngeneic HSCT group (8.3% vs 28% at post-transplantation week 6 and 17% vs 43% at post-transplantation week 16); however, the relapse rate at 38 weeks was similar between the two groups (83.3% vs 85.7%). Adding donor splenocytes, containing reactive T-cells, dramatically decreased the relapse rate, such that the relapse rate was only 20% at post-transplantation week 38, suggesting a GVT effect. This GVT effect was accompanied by a severe GVH effect, and OS was not different between the allogeneic BM + splenocyte and the syngeneic HSCT groups. In an attempt to induce a GVT effect without a severe GVHD, we transplanted allogeneic Treg cells along with allogeneic BM, however, survival and relapse rates were similar to those with allogeneic BM only. Taken together, these findings suggest that a lethal dose of ionizing radiation (1000 rads) is insufficient to eradicate the MDS initiating cell, and that transplantation of donor CD3+ splenocytes leads to decreased relapse rates, but at the cost of severe GVHD. We suggest that the NHD13 mice are a feasible pre-clinical model for the study of HSCT for MDS.

P-49: methyl2cnvDX: An optimized algorithm to derive genomic copy number status from Illumina Infinium 450k array data on clinical FFPE pathology samples

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The underlying molecular basis of the disease phenotype is complex, and may potentially involve errors or failure of any cellular component or biological process, as reflected in gene expression levels, epigenomic patterns, and DNA sequence, structural, and copy-number variants. Gene copy number changes could cause disturbance of inherited gene copy number balances and lead to altered gene expression levels or post-transcriptional protein levels. DNA methylation programming has roles in maintenance of gene expression profiles and cell state. Microarray technologies including array CGH and methylation profiling permit the exploration of molecular alterations genome-wide. Ideally, we could perform all available technologies on clinical specimens for discovery, but practically, we may face outside constraints, where analyses are not technology-limited, but rather, specimen-limited (only enough mass for the input requirements for one assay), or budget-limited (only enough funds for limited profiling). In our lab, we have developed a method to re-

cycle methylation data from Illumina Infinium 450k arrays to generate array CGH-like copy number patterns on clinical samples, focused on the unique algorithmic optimization for formalin-fixed, paraffin-embedded (FFPE) tissues. Our results suggests that the optimized CGH patterns generated from methylation data are comparable to that from array CGH, and provide a useful tool to more efficiently and economically study human diseases.

P-50: Identify miR-23a target gene connexin-43 (Cx43 /GJA1), as a mediator of intercellular signaling critical to osteoblast development in osteosarcoma

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Osteosarcoma is the most common type of bone cancer in children and adolescents.Impaired differentiation of osteoblast cells is a distinguishing feature of this aggressive disease. As improvements in survival outcomes have largely plateaued, better understanding of the bone differentiation program may provide new treatment approaches. To this end, we carried out a large-scale integrative computational analysis of mRNA and miRNA expression as well as genome copy number aberrations of Forty-seven of osteosarcoma and twenty osteoblast cell lines. This work identified copy number gain and over-expression of miRNA cluster miR-23a_27a_24-2 in a substantial fraction of osteosarcoma samples. Previous studies identified interactions between the microRNAs in this cluster, particularly miR-23a, and select genes important for bone development. However, global changes in gene expression associated with functional gain of this cluster have not been fully explored. Experimental results show that over-expression of miR-23a delays ossification and calcification in osteosarcoma (HOS) cells. Downstream bioinformatic analysis identified miR-23a target gene connexin-43 (Cx43 /GJA1), a mediator of intercellular signaling critical to osteoblast development, as acutely affected by miR-23a levels. Connexin-43 is up-regulated in the course of HOS cell differentiation and is down-regulated in cells transfected with miR-23a. Analysis of gene expression data, housed at Gene Expression Omnibus, reveals that Cx43 is consistently up-regulated during osteoblast differentiation. Suppression of Cx43 mRNA by miR-23a was confirmed in vitro using a luciferase reporter assay. This work demonstrates novel interactions between microRNA expression, intercellular signaling and bone differentiation in osteosarcoma

P-51: Analysis of a dataset of 207 rectal cancer samples

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As the 3rd most commonly diagnosed cancer, colorectal cancer makes up of about 10% of all cancer cases and afflicts 1.3million patient each year. It is also the 3rd leading cause of cancer death. Every year more than 50,000 patients die of this disease. To analyze the molecular mechanism of rectal cancer, we explore the gene expression of cancer samples of 207 patients; most of them have paired control. We also study SNPs as well as prognosis data of these patients. Rectal cancer samples show a significant downregulation of genes in metabolic pathways. The general expression pattern separates cancer samples into ÃČÂŠcontrol likeÃČÂŞ subgroup and ÃČÂŠcancer likeÃČÂŞ subgroup. There is no significant prognosis difference between these two groups. We observe a strong correlation between the average gene expression and the copy number change of each chromosome of cancer samples. Several SNPs shows differential survival among patients with different alleles. Clinical data shows that these SNPs affect prognosis independent of lymph node status.

P-52: Single Cell RNA Transcripts Analysis

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Advanced sequencing technologies have demonstrated not only intertumor heterogeneity among individual cancer patients, but also provided increasing evidence of intratumor genetic heterogeneity (ITH) within indi-

vidual tumor biopsies. Preexist tumor subclones within ITH may ultimately influence the natural progression, therapeutic response, drug resistance and tumor recurrence. However, such clinically important information of rare subclones may not be always revealed by conventional gene methods when tumor tissue used as starting material. Here, we present the methods and preliminary data on RNA transcripts analysis of single prostate cancer cells. Using Fluidigm C1TM Single-Cell AutoPrep System to capture and generate cDNA from single cells, followed by High throughput Real time PCR analysis using Fluidigm 48.48 Dynamic Array platform on a BioMark system, we show that the established procedure is capable of accurately profiling gene expression of single cells. Furthermore, the data exhibit high fidelity across transcripts in a broad range of abundance levels. Importantly, it opens the possibility to conduct single cell RNA Seq when combined with SMARTer Ultra Low RNA for Illumina Sequencing kit (Clontech) for cDNA library preparation. As a conclusion, it is feasible to perform gene expression and sequencing analysis at single cell level and single cell genetic analysis could potentially reveal true diversity of cancer cells and provide valuable information for clinical application.

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